THE EFFECT OF THE SULPHYDRYL COMPOUNDS ON MILK PRODUCTION [1]

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TWO FIGURES

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Daggs and Tomboulian ('35) have shown the stimulating effect of various dietary substances on milk production in the normal albino rat. The dietary principle that augmented normal lactation in rats receiving the necessary vitamins and minerals was limited to the protein fraction. The fact that the principle was not lost upon autolyzing liver showed it to be associated with protein degradation products. The protein degradation products, extracts and amino acids that contained the lactation principle all had a relatively high sulphur content. That this sulphur had to be organic was indicated by the fact that potassium sulphide had no effect whereas cystine had a very marked effect. It was their belief that the sulphydryl compounds in some way afforded the active principle in augmenting milk production by dietary means. The present study involves further proof of that belief.

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[2] Submitted by Miss Lidfeldt as partial fulfillment of the requirements for the master of science degree, University of Rochester.

The method used was the same as that reported by Daggs ('35). The rats were maintained on a stock diet of Purina Chow until a few days before parturition when they were placed in special cages and given experimental diets. All litters were limited to six young which were weighed en mass every day. Only 'perfect' litters were used in calculating the results. 'Perfect' litters refer to experiments where the mother has shown no signs of ill health and has successfully cared for and weaned all six young allotted to her at parturition. The lactation indices were calculated from the slopes of the logarithmic growth curves of the suckling young. K_1 is the slope of the logarithmic growth curve from the fourth to the tenth day and K_2 that of the curve from the tenth to the seventeenth day after birth. The addition of K_1 and K_2, disregarding the decimal point, gives the 'lactation index'—merely a numerical means for comparing results. (See the above quoted papers for the details of the method and the care of the animals.)

Table 1 gives a composite of the diets used in the present study. They were fed ad lib. with the exception of the daily supplements.

The results obtained by the use of the various diets are listed in table 2. The diets are all designated by their sources of protein, amino acids, etc. The other constituents are essentially the same in all diets unless otherwise indicated. The protein level was kept at a minimum so as to determine better the difference between various protein sources since a high protein diet in itself augments lactation. Casein (15) was used as a basal diet, the (15) referring to the parts of casein in the diet. Comparisons were made to this basal ration which gives a lactation index of 760.

Daggs and Tomboulian ('35) cited three experiments on a mixture of cystine, glycine and glutamic acid. (It is known that the body is capable of making glutathione from these three amino acids.) From these few experiments the indication was that the simulated glutathione diet was superior to the cystine diet. When more experiments on this diet were

TABLE 1

Composition of diets

DIETS	CASEIN (15)	CYSTINE	CYSTINE GLYCINE GLUTAMIC	METHIONINE	CYSTEINE	CYSTEINE GLYCINE GLUTAMIC	LYSINE	TAURINE	Na TAURO-CHOLATE	CYSTINE (2) CHOLINE (1)	CYSTINE (5)	GLYCINE	GLUTAMIC	CYSTINE (2)	S-BENZYL-CYSTEINE
Casein	15	14	12	14	14	12	14	14	12	12	10	14	14	13	14
Salt mixture[1]	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Agar agar	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Starch	76	76	76	76	76	76	76	76	76	76	76	76	76	76	76
Lard	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14
l-cystine		1	1							2	5			2	
Cysteine-HCl					1	1									
Glycine			1			1						1			
d-glutamic acid			1			1							1		
dl-lysine-HCl							1								
dl-methionine				1											
Taurine								1							
Na taurocholate									3						
Choline-HCl										1					
S-benzyl-cysteine[2]															1

Wheat germ oil, 5 drops per rat per day.
Cod liver oil, 6 drops per rat per day.
Yeast (dried), 3 gm. per rat per day.

[1] Modified Osborne and Mendel salt mixture (see Daggs, '35).

[2] Made by Dr. C. E. Braun, department of organic chemistry, University of Vermont.

completed, the average lactation index was slightly lowered so that it seemed probable cystine was just as effective as the cystine, glycine, glutamic acid mixture. This is a reasonable assumption since the body can easily supply the glycine and the glutamic acid but not the cystine. This finding indicated that the S-H group was the essential factor. To substantiate this claim various S-H compounds were tried as well as the separate amino acid constituents of glutathione. In order to be sure that the cleavage of cystine to cysteine by the body did not hamper the results the three amino acids composing

TABLE 2

Lactation indices of sulfur-containing compounds

	K_1	K_2	LACTATION INDEX	NUMBER OF LITTERS
Casein (15)	0.0488	0.0272	760	9
Cystine glycine glutamic	0.0588	0.0343	931	18
Cystine	0.0586	0.0350	936	6
Glycine	0.0510	0.0260	770	7
Glutamic	0.0504	0.0244	748	9
Cysteine glycine glutamic	0.0595	0.0337	932	11
Cysteine	0.0579	0.0363	942	10
Methionine	0.0577	0.0358	935	8
Lysine	0.0475	0.0246	721	4
Cystine (2)	0.0550	0.0340	890	13
Cystine (5)	0.0475	0.0253	728	7
Taurine	0.0565	0.0258	823	10
Cystine (2) choline (1)	0.0560	0.0333	893	9
S-benzyl-cysteine	0.0460	0.0238	698	5

glutathione, namely cysteine, glycine and glutamic acid, were tried together and singly. The lactation index (932) for the cysteine, glycine, glutamic acid mixture was identical with that (931) found for cystine, glycine and glutamic acid. Cystine, index 936, is just as effective as cysteine, index 942. Glycine and glutamic acid showed no increase over the basal giving indices of 770 and 748, respectively. These facts seem to prove the point that the S-H group is the essential factor. Since methionine is capable of replacing cystine in growth studies it was assumed that it might play a similar role in

these studies. Methionine proved to be equally as efficient as cysteine giving indices of 935 and 942, respectively.

It has been argued that the better rate of growth shown by young suckling a cystine fed mother was due to the cystine passing directly into the milk thus having its effect upon the growth processes in the young. To further substantiate the negative results obtained by feeding cystine directly to the suckling young (Daggs and Tomboulian, '35, p. 590) lysine, another growth stimulating amino acid, was fed to lactating rats. Lysine showed an index of 721 proving its ineffectiveness in stimulating lactation under the conditions of this experiment. More detailed experiments on the relation of lysine to lactation are in progress. When poorly lactating human mothers were fed cystine their milk, when analyzed by Okuda's method, showed no greater amounts of the S-H group than did human milk taken from mothers not fed cystine. This again substantiates the claim that the S-H group does not act by passing into the milk and affecting the growth of the young directly.

The next step was to obtain some idea of the extent to which the cystine content of the diet could be raised before becoming ineffective or toxic. When 2 parts of cystine (cystine (2) table 2) were used very little effect was lost whereas 5 parts of cystine (cystine (5)) did prove quite ineffective (index 728). One part of cystine in the diet is evidently optimal.

It was desirable to try to gain some knowledge of the action of the S-H group in stimulating lactation. The first step in the catabolism of cystine is its reduction to cysteine. This easily takes place within the body making available the S-H group. The next step is thought to be an oxidative deamination, the ammonia going to urea and the sulphur eliminated mostly as inorganic sulphate. However, some of the sulphur may be eliminated in the neutral sulphur fraction. Taurine is thought by some to be an intermediate in the metabolism of cysteine. That this reaction occurs in the body is not established. Lewis ('35) has suggested that the formation of taurocholic acid in the body occurs subsequent to the conjugation of cholic acid with cystine. Taurine is oxidized with

difficulty if at all (Schmidt and Clark, '22). The fact that taurine cannot replace cystine for growth has been shown by many. Even though these findings point to the fact that taurine probably is not directly concerned in the oxidation of cysteine in the body it was tried in these lactation studies because the sulphur mechanism concerned in lactation may not be the same as that for growth. It will be remembered that taurine definitely cannot replace cystine for growth. Taurine gave a lactation index of 823 showing that it did augment lactation some but not nearly so well as the S-H compounds. Since the place of taurine in the intermediary metabolism of cysteine is not well established, it is difficult to explain its function in lactation.

Lewis ('35) points out that when S-substituted derivatives of cysteine are used, thus eliminating the presence of a free S-H group, oxidation in the body does not occur. S-benzyl-cysteine is a compound wherein the sulphur is held in a still different combination than either that of methionine ($S\text{-}CH_3$) or taurine (SO_3H). S-benzyl-cysteine when fed to lactating rats gave an index of 698 showing it to be definitely inactive in augmenting lactation.

Bennett ('37) quotes Hammett as postulating that an increase in the state of oxidation of S-H means a decrease in its reversible reduction capacity. Bennett ('37) has found that l-cystinedisulphoxide (-SO-SO-) will replace cystine for growth, but l-cysteinesulphinic acid ($-SO_2H$) will not, thus partially confirming Hammett's prediction. When S-(guanylthio)-cysteine-2HCl (-SOH) was used some replacement occurred. This is explained by Bennett as probably due to the cystine formed from S-(guanylthio)-cysteine-2HCl since it is theoretically capable of yielding 33 to 50% cystine. Taurine is partially utilizable for lactation but not for growth. Therefore, it appears that the S-H derivative necessary for lactation can be of a higher oxidized nature than that necessary for growth. Before this can be definitely settled other derivatives similar to the compounds Bennett used should be tried in lactation studies.

The statement made by Lewis ('35) concerning S-H compounds and growth, i.e.,

the peculiar nutritional role of the three sulphur containing amino acids which appear to be interchangeable in growth promotion is not simply a function of the disulphide (S-S) or sulphydryl (S-H) group alone, but is related to the structure of the molecule as a whole. Only the S homologues containing an alpha amino group and three or four carbon atoms appear to possess significant biological properties . . . .

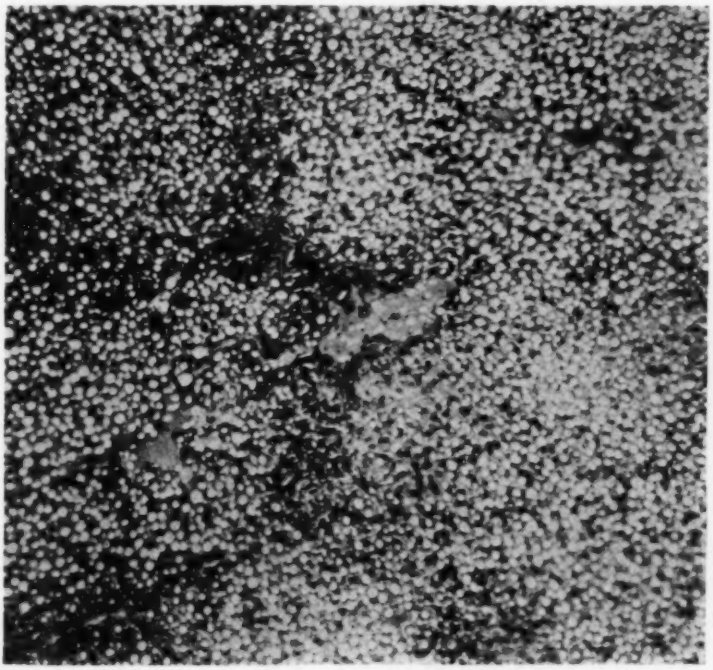

Fig. 1 Section of liver from a lactating rat fed the cystine (2) diet.

does not appear to hold entirely for the sulphur containing compounds stimulating lactation unless the rat is capable of converting some taurine back into cysteine. This, however, seems highly improbable (Muldoon, Shiple and Sherwin, '24).

If taurine stimulates lactation perhaps taurocholic acid or sodium taurocholate would retain some lactation stimulating effect. When sodium taurocholate was tried it proved a failure. Therefore in the conjugation of taurine with cholic acid to form taurocholic acid the available lactogenic property of taurine is destroyed.

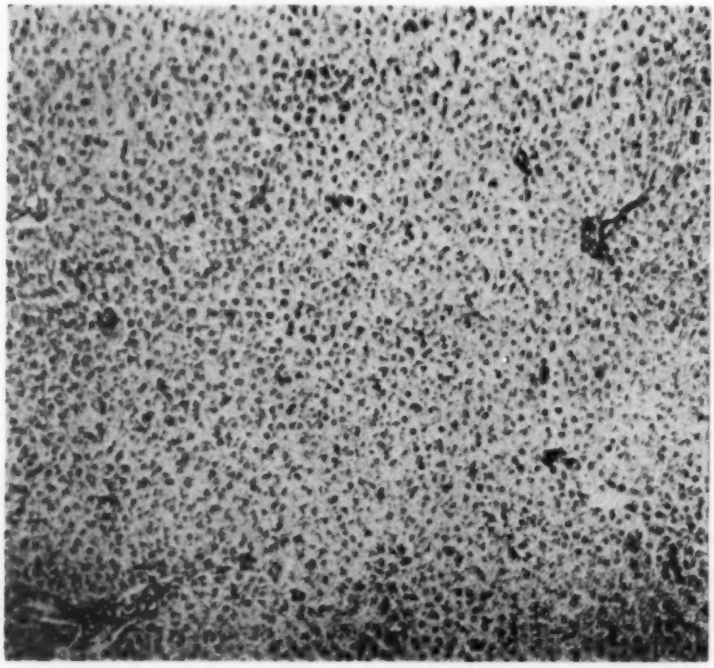

Fig. 2 Section of liver from a lactating rat fed the cystine (2) choline (1) diet.

It was noticed that the livers of the animals fed cystine (2) and cystine (5) diets appeared enlarged and milky. Knowing of the work of Beeston and Channon ('36) it was assumed that these livers would show a marked fatty infiltration on histological examination. This proved to be the case. Figure 1 shows a liver from an animal fed cystine (2). Several

facts have indicated that the liver is probably involved in the lactation process. It is the best natural protein source of the lactation principle (Daggs, '31). Mapson ('32) has shown it to contain a lactagogue and Nakahara, Inukai and Kato ('34) claim to have extracted a potent lactagogue called 'factor L.' Since cystine stimulates lactation and also causes fatty changes in the liver perhaps these changes are essential to the lactation stimulating mechanism. Best and Channon ('35) have shown that choline will prevent fatty livers. When 1 part of choline was fed along with cystine (cystine (2), choline (1)) the lactation index was 893, identical with that for cystine (2), (890), showing that the prevention of the fatty liver did not limit the lactation stimulating effect of cystine. Figure 2 shows a section of liver from a rat on cystine (2) choline (1).

Several animals on each diet were killed at the end of the seventeenth day following parturition. The following tissues were examined histologically: liver, kidney, adrenal, pituitary and mammary gland. With the exception of the aforementioned changes in the liver no abnormalities could be detected. Although many of the mammary glands from cystine (2) fed animals showed a greater number of proliferating follicles and fewer resting alveoli even at the periphery than normals, it did not seem markedly significant since sections from glands of rats on stock ration showed wide variations in appearance.

CONCLUSIONS

1. The sulphydryl amino acids existing either as such (cysteine) or in the potential form (cystine, methionine), are dietary lactogogues.

2. The simulated glutathione, that is, both the cystine, glycine and glutamic acid diet and the cysteine, glycine and glutamic acid diet, each acts as a lactagogue parelleling the effect of cystine or cysteine alone.

3. The non-sulphur amino acids of the tripeptide glutathione, glycine and glutamic acid, have no lactogenic action.

4. In the intermediary metabolism of the sulphydryl compounds, the lactogenic effect is not entirely destroyed at the taurine stage.

5. The catabolic pathway of taurine to form taurocholic acid in the process of milk production is improbable.

6. It is not possible to surpass the animal's hereditary capacity for lactation by increasing the level of the sulphur amino acid content of the diet.

7. It is very improbable that the effect of the sulphydryl group is through the liver, for there is no relationship between fatty infiltration of the liver and milk production.

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THE PARALYSIS IN THE YOUNG OF VITAMIN E DEFICIENT FEMALE RATS [1]

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ONE PLATE (FOUR FIGURES)

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A specific nutritional disease in the young of female rats on vitamin E deficient diets was first described by Evans and Burr ('28). On or about the twentieth day of life the young animals developed a paralysis, especially of the hind quarters. Many of the afflicted rats died, some recovered completely, and a few lived but remained partially paralyzed through life. These investigators showed that the early administration of vitamin E concentrates to the young or to their mothers prevented the onset of the symptoms.

Some anatomical and histological changes in the nerves of such paralyzed animals have been described by Lipshutz ('36). He observed degenerated nerve tracts in the cord which he considered responsible for the paralysis.

The possibility that there might be some relationship between this disease and the muscular lesions which appear in guinea pigs and other Herbivora on purified diets (Goettsch and Pappenheimer, '31; Woodward and McCay, '32; Madsen, McCay and Maynard, '33; Victor, '34) suggested a histological study of the muscles of the paralyzed rats. The nervous systems of the same animals have also been carefully examined.

[1] Presented in part before the thirty-first meeting of the American Society of Biological Chemists at Memphis, April 21, 1937 (Proc. Am. Soc. Biol. Chem., J. Biol. Chem., 119, xxiv, 1937).

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EXPERIMENTAL

The basic vitamin E deficient diet used contained:

	%
Casein	18
Sucrose	45.5
Lard	22
Yeast [3]	8
Salts [4]	4.5
Cod liver oil	2

It is adequate for growth and, with vitamin E supplementation, for reproduction, but not for lactation except in a few cases (approximately 5%) even though dry yeast is offered ad libitum to the lactating animals. Of the litters which did survive, less than half developed the paralytic disease.

Young rats developing the paralytic symptoms were carefully watched. When the condition became so acute that death was expected within 12 hours (23 to 36 days of age), they were killed and the skeletal muscles and nerves were removed for histological study. Figures 1 to 3 are photomicrographs of sections from the leg muscles of paralyzed rats. It is readily apparent that the muscles are badly damaged. There is a marked proliferation of the nuclei and necrosis of the muscle fiber (fig. 1). In some cases hyaline necrosis of the fibers could be observed (fig. 2). Accompanying these changes at times was a severe cellulitis with numbers of inflammatory cells scattered through the inflamed area (fig. 3). Such muscle damage in greater or less degree has been observed in every animal which showed any external symptoms of leg weakness or paralysis. Figure 4 is a section of the muscle from a normal rat 22 days old.

Skeletal muscles from various sections of the body all showed the same changes. Histologically, at least, the heart muscle was not affected. As a control experiment, a part of one of the sciatic nerves was removed from eighteen rats at varying ages from 1 to 20 days. The muscles of these animals were then examined at 23 or 26 days of age. The

[3] Courtesy of Northwestern Yeast Company.

[4] Hawk and Oser ('31), Science, vol. 74, p. 369.

sections demonstrated the atrophic fibers and nuclear proliferation characteristic of this type of lesion but in no case did they show the marked necrosis of individual fibers, cellulitis, etc., which characterized the muscles of vitamin E deficient young. Contrary to Lipshutz's observations, no abnormalities in the nerves, cord, or brain could be observed.[5]

DISCUSSION

The sudden occurrence of the paralysis in second generation rats in a very specific age group is a baffling phenomenon. Sections from muscles of first generation animals, fed vitamin E deficient diets from weaning and for several months (Olcott and Mattill, '37) showed no degenerate fibers.[6] On the other hand, second generation animals which escaped the paralytic symptoms (presumably because their stores of vitamin E were sufficient to protect them through the critical period), never developed symptoms later in life although they were maintained on the vitamin E-deficient regime. Sections of muscles obtained from these rats showed occasional necrotic fibers.

The paralysis resembles that observed by Pappenheimer and Goettsch and others in guinea pigs and rabbits in that the lesions are primarily muscular and do not involve the nervous system (Rogers, Pappenheimer and Goettsch, '31; Victor, '34). The histopathological condition of the muscles is also strikingly similar.[7]

Goettsch and Pappenheimer ('31) showed that vitamin E concentrates alone did not prevent and could not cure the so-called nutritional muscular dystrophy in guinea pigs. Nevertheless, all of the effective foodstuffs which they investigated contained vitamin E. Morgulis and Spencer ('36)

[5] Nissl, Marchi and haematoxylin-eosin stains were used in examining the nervous systems.

[6] We were not able to duplicate the results of Ringsted ('35) whose rats became paralyzed after 18 to 22 weeks on a vitamin-E deficient ration. Burr, Brown and Mosely ('37) have recently demonstrated paralytic symptoms in rats after 22 months on a diet in which vitamin E was not supplied.

[7] Comparison made with sections from the muscles of guinea pigs fed Pappenheimer and Goettsch's diet 13 by Prof. H. M. Hines of the department of physiology.

have recently demonstrated that at least two independent factors are required. The analogous pathological picture seen in rats suggests, as do Morgulis and Spencer, that one of these factors may be vitamin E.

If this hypothesis is correct, it would furnish a possible explanation for results obtained by Madsen ('36), who showed that the inclusion of cod liver oil in diets fed to Herbivora markedly increased the incidence and severity of the disease. The oxidative reactions initiated by the autoxidation of cod liver oil are destructive to vitamin E (Cummings and Mattill, '31) and the diets containing cod liver oil possibly had much less of this vitamin than those from which cod liver oil was omitted.

I am grateful to Dr. H. P. Smith and his staff in the department of pathology and to Dr. C. G. Barer of the department of neurology for their help in the preparation and interpretation of the tissue sections.

SUMMARY

The paralysis in the young of vitamin E deficient female rats is due to lesions of the skeletal muscle very similar in character to those observable in the so-called nutritional muscular dystrophy of Herbivora.

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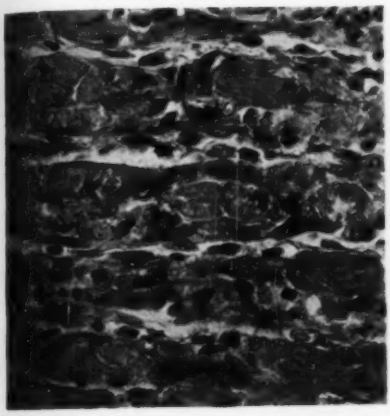
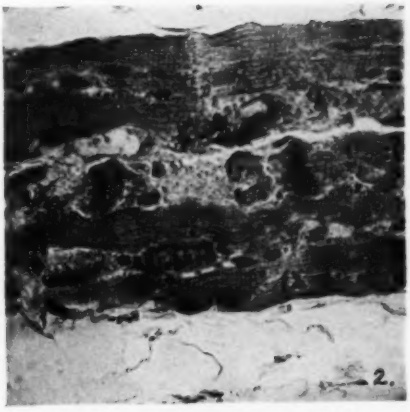
PLATE 1

EXPLANATION OF FIGURES

1 to 3 Sections of gastrocnemius muscles from paralyzed animals.

4 Section of normal rat gastrocnemius muscle. Slides stained with hematoxylin and eosin. × 200.

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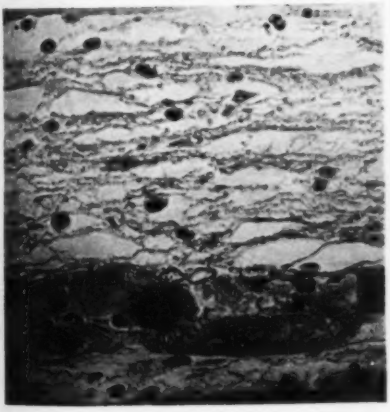

THE EFFECTS OF SMALL AMOUNTS OF ETHYL ALCOHOL ON THE RESPIRATORY METABOLISM OF HUMAN SUBJECTS DURING REST AND WORK

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Our knowledge of the part that ethyl alcohol is capable of playing in normal metabolic processes has been considerably clarified by the work of Mitchell ('35). He has shown that when ethyl alcohol is added to a complete diet growth is accelerated and the retention of both nitrogen and fat increased. He concludes that "the energy of ethyl alcohol is to a large extent available for physiological purposes." Mitchell's work, however, gives no direct evidence as to the effect of muscular exercise on the metabolism of alcohol. The earlier investigators, notably Atwater and Benedict ('02), Mellanby ('19) and Sommerkamp ('24), concluded, as a result of their investigations, that the energy of alcohol was available for the performance of muscular work. On the other hand the more recent investigations of Carpenter, Lee and Burdett ('33), Canzanelli, Guild and Rapport ('34) and Nyman and Palmlöv ('34) seem to show that muscular exercise has no effect upon the rate of oxidation of ethyl alcohol. It should be pointed out, however, that Carpenter, Lee and Burdett used but one human subject and that the conclusions of Canzanelli, Guild and Rapport are based on observations made on a single dog. Furthermore, from a theoretical aspect such conclusions are difficult to explain in view of the fact that alcohol seems to be present normally in body tissues (Gettler, Niederl and Benedetti-Pichler, '32), and that it is

usually postulated as an intermediary product in the normal metabolism of carbohydrates (Grafe, '33). Mitchell's demonstration of the ability of ethyl alcohol to produce growth would seem to justify the conclusion that alcohol could be used at least indirectly in the performing of muscular work since he has proved that a part of its energy can be stored in the animal organism. Carpenter ('33) has reviewed this subject and concludes "that the literature by no means furnishes adequate data to draw a decisive conclusion in regard to whether or no muscular activity accelerates the combustion of alcohol."

METHODS

We have investigated the effects of small amounts of ethyl alcohol on the respiratory exchanges during rest and work. Two series of experiments have been carried out using five different subjects upon whom a total of more than fifty tests were run. The oxygen consumption, carbon dioxide production and total respiratory volume were determined by means of the Tissot-Haldane technic. Expired air was collected in the Collins chain compensated gasometer. For basal and resting tests spirometers of 100 liters capacity were used while in the work tests a double chain compensated gasometer of 700 liters capacity was used. Samples of expired air were analyzed in the Boothby modification of the Haldane gas analysis apparatus (Boothby and Sandiford, '20). This apparatus was checked by frequent analyses of outdoor air. For the work test a bicycle ergometer of the Prony brake type (manufactured by Warren E. Collins) was used.

In the first series of experiments three subjects were used. With these subjects both control and alcohol tests were run while the subject was lying in a basal condition, while he was sitting at rest on the bicycle, and while he was pedalling the bicycle at a rate which was equal to 2500 to 3000 foot pounds of work per minute. Following the work period the expired air was collected during a recovery period of 35 minutes.

The arrangement of the spirometers was such that the collection of expired air was continuous during work and recovery, none of it being allowed to escape. In basal tests the procedure was as follows: the subject reported to the laboratory in a post-absorptive condition and rested on a comfortable bed for the usual ½-hour period. One or two basal metabolism determinations were then made. The subject then ingested 200 cc. of water if the test was a control test, or 200 cc. of approximately 10% alcohol in alcohol tests. (The quantity of alcohol given was from 0.25 to 0.30 gm. per kg. of body weight). The respiratory metabolism was then determined at ½-hour intervals for approximately 2 hours.

In the work tests the subject came to the laboratory in a post-absorptive condition and ingested the water or alcohol solution according as the determination was to be a control or alcohol test. The dosage of alcohol was the same as that used in basal tests. The subject then sat quietly on the bicycle ergometer for 15 to 20 minutes after which two determinations of the respiratory exchanges during rest were made. The values obtained in these tests were used as a base line from which the excess metabolism of work was calculated. After the usual washout periods the respiratory exchanges were measured for a 10-minute work period and a 35-minute recovery period.

In the second series of experiments no basal tests were run. Approximately the same quantity of alcohol was ingested but in this case it was diluted with 200 cc. of a 10% dextrose solution. Control tests were run in this series 1) in which 200 cc. of water was ingested and 2) in which 200 cc. of 10% dextrose was given. It seemed desirable to test the effects of alcohol plus dextrose upon the respiratory exchanges during rest and work since the recent work of Carpenter and Lee ('37) indicate that the metabolism of alcohol is considerably accelerated if it is ingested with glucose. Haggard and Greenburg ('37) have also shown recently that the toxic effects of alcohol are greatly decreased if the blood sugar is elevated. Both of these investigations seems to indicate

an intimate relationship between the metabolism of ethyl alcohol and that of dextrose. In all other respects the experiments in this second series were similar to those of the first series. Three subjects were used in this series of experiments but one of these had already served in the first series and therefore the total number of subjects was five.

RESULTS AND DISCUSSION

The results of the experiments on basal metabolism carried out in the first series are shown in table 1. In tabulating our results we have shown only averages. The degree of variation between individual determinations has been indicated by the standard deviation which in each case follows the tabulated average under the heading SD. This makes it a simple matter to compare the degree of variability obtained in individual tests with the average difference between control and alcohol tests. The oxygen consumption was apparently affected by the ingestion of alcohol only slightly, if at all. With two of the subjects there is no significant change while in the third subject there was an increase of about 6%. However, with this subject (JRM) there was but one basal test preceding the administration of alcohol which makes this apparent rise of little significance. This is all the more true in view of the fact that the oxygen consumption during control tests is approximately the same as that following the ingestion of alcohol.

All three subjects show a noticeable drop in the R.Q. following the ingestion of alcohol. This is 0.04 in the case of FAH. The variability obtained in the tests following alcohol is considerably less than that of any other series in the case of both subjects FAH and JRM. The drop of 0.02 which occurred in the R.Q. of subject RCG following the ingestion of alcohol is of doubtful significance since in control tests a spontaneous drop even greater than this occurred. The results obtained on FAH may justly be considered of greater significance than those on RCG and JRM since the total number of tests on him was about twice as great as on

TABLE 1

The effect of alcohol on the basal oxygen consumption respiratory quotient and ventilation equivalent

SUBJECT	TESTS		BEFORE						Number of tests	AFTER					
	Type	Number	Cubic centimeters O_2 per minute	SD.	R.Q.	SD.	Ventilation equivalent O_2 [1]	SD.		Cubic centimeters O_2 per minute	SD.	R.Q.	SD.	Ventilation equivalent O_2 [1]	SD.
FAH	C.	8	264.6	1.8	0.82	0.001	1.92	0.03	12	260.6	2.8	0.81	0.001	2.04	0.03
	A.	7	266.0	2.8	0.83	0.001	1.89	0.02	22	265.4	1.3	0.79	0.003	1.99	0.02
RCG	C.	4	186.5	5.2	0.82	0.016	1.88	0.03	5	188.6	2.2	0.79	0.011	1.92	0.03
	A.	3	188.2	3.4	0.82	0.014	1.93	0.01	7	187.6	1.7	0.80	0.012	1.96	0.02
JRM	C.	4	230.5	1.1	0.79	0.012	2.24	0.10	5	226.0	1.3	0.81	0.016	2.26	0.09
	A.	1	214.5		0.84		2.12		4	227.1	2.0	0.82	0.006	2.27	0.04

[1] Liters of air exhaled per 100 cc. of oxygen absorbed.

either of the other two subjects. If with this subject we take the drop in the quotient as a measure of the amount of alcohol being oxidized, we calculate that during the 2 hours immediately following the taking of the alcohol this substance was oxidized at the rate of about 30 mg. per minute.

In any study of respiratory exchanges a knowledge of the ventilation volume is of major importance since it is generally agreed that fluctuations in the total quantity of air breathed have a marked effect upon the excretion of carbon dioxide. Recording the ventilation volume is not entirely satisfactory since this does not take into consideration the fluctuations in oxygen absorption. We have therefore chosen to use as our criterion of changes in ventilation the ventilation equivalent for oxygen as defined by Knipping and Moncrieff ('32). This is the volume of air exhaled per 100 cc. of oxygen absorbed. These authors give 2.37 liters as the average figure for their normal subjects with a variation of approximately 25%. It will be seen from table 1 that the ventilation of our subjects as indicated by the ventilation equivalent was quite normal. There was a tendency in all subjects for the ventilation to increase slightly in the second half of the test. This tendency was slightly less marked in alcohol tests than in control tests.

We may sum up our results on basal tests with the statement that there was no indication of any specific dynamic action of alcohol during a period of 2 hours after its ingestion. There was no significant change in ventilation but there was a significant drop in the R.Q. which may be interpreted as an indication that alcohol is being oxidized. The validity of this last assumption is discussed later in this paper.

In table 2 we have recorded the oxygen consumption and ventilation equivalents during rest and work in all of the experiments of both series. It will be noticed by comparing the resting oxygen consumption of RCG, FAH and JRM, with the figures obtained in basal tests on these subjects, that sitting quietly on the bicycle caused a rise of from 5 to 8% in the oxygen consumption. Furthermore, the results obtained on these same subjects again fail to show any specific

dynamic action of alcohol. It may be, however, that more time is necessary for alcohol to produce its stimulating effect than was allowed in these tests. Two of the three subjects showed a slight drop in resting oxygen consumption after the ingestion of alcohol. Since both of these subjects reported subjective effects of sleepiness and general relaxation, it may

TABLE 2

The effect of dextrose and alcohol on the oxygen consumption and ventilation equivalent during rest and work

SUBJECT	TESTS		REST				WORK			
	Type	Number	Cubic centimeter O_2 per minute	SD.	Ventilation equivalent O_2[1]	SD.	Cubic centimeter O_2 per minute	SD.	Ventilation equivalent O_2[1]	SD.
RCG	C.	5	199.8	3.0	2.17	0.09	1031	59	2.10	0.04
	A.	5	196.2	4.9	2.20	0.10	950	36	2.05	0.04
	D.	3	206.7	4.1	2.33	0.18	1034	35	2.19	0.04
	D. + A.	3	209.1	1.9	2.08	0.17	951	36	2.11	0.01
FAH	C.	7	283.0	6.1	1.95	0.05	1335	46	1.99	0.04
	A.	7	286.5	5.7	1.79	0.01	1317	48	1.97	0.04
JRM	C.	3	252.0	0.7	2.46	0.01	1216	20	2.08	0.04
	A.	3	241.9	4.8	2.65	0.07	1206	56	2.10	0.01
CAV	C.	2	240.1	4.9	2.16	0.09	1143	56	2.10	0.02
	D.	2	266.8	7.6	2.03	0.13	1196	120	2.12	0.03
	D. + A.	3	255.4	5.4	2.01	0.11	1261	43	2.10	0.02
DUN	C.	2	246.9	3.8	2.47	0.11	1014	56	1.99	0.01
	D.	3	254.4	0.3	2.45	0.04	1087	36	2.03	0.04
	D. + A.	4	260.6	5.2	2.28	0.04	1133	41	1.97	0.03

[1] Liters of air exhaled per 100 cc. of oxygen absorbed.

be that this slight decrease in oxygen consumption is an indication of the narcotic effect of the alcohol. All three of the subjects that received dextrose showed a definite specific dynamic action following the ingestion of this substance. This amounted to about 4% in the case of RCG and DUN and about 8% with CAV. It is further significant that

the increase in oxygen consumption following the ingestion of dextrose plus alcohol was approximately the same in all three of these subjects as that obtained after the ingestion of dextrose alone. Thus it appears that alcohol had no specific dynamic action on any of our five subjects and we must conclude that if alcohol is capable of producing such an effect it must do so after an interval of more than 2 hours.

The oxygen consumption for work is characterized by an unusually high degree of variability. This is the result of a certain amount of unavoidable variation in the amount of work done during different tests. It is noticeable particularly in the case of RCG that the amount of oxygen used during work periods after alcohol and after dextrose plus alcohol, is nearly 10% less than the oxygen consumption during control tests. This is probably the result of a decreased amount of work performed in alcohol tests as a result of drowsiness brought on by the narcotic effect of the alcohol.

Ventilation equivalents show that there was no tendency toward over-ventilation in these tests since they are no higher for sitting at rest than they were in the basal tests. All of them except for the subjects JRM and DUN are lower than the average figures for normals given by Knipping and Moncrieff ('32). It is also worthy of comment that no rise in the ventilation equivalent occurred in the work periods. In fact in most cases there was a slight drop in the figure which would indicate that during the work periods oxygen was absorbed from the respired air more efficiently than at other times. Alcohol seems to have slightly decreased the ventilation equivalent in the cases of FAH, CAV and DUN. With subject RCG the administration of dextrose plus alcohol also produced the lowest ventilation equivalent ever observed on this subject. This effect is probably due to a depression of the respiratory center by the alcohol. The high degree of variability shown in the ventilation equivalents during rest, as indicated by the standard deviations given in the table, probably indicates that this center is easily affected by extraneous and fortuitous sensory impulses. The relatively

low degree of variability found in the ventilation equivalents during work would indicate that during periods of increased utilization of oxygen the respiratory center ceases to respond to such extraneous and fortuitous stimuli and is controlled almost entirely by the physiological needs of the organism.

Since in the latter part of this paper we shall be concerned largely with respiratory quotients and their interpretation it is important to know what, if any, changes in alveolar carbon dioxide occurred in the course of these experiments. No determinations of alveolar CO_2 were made in the first series

TABLE 3

The effect of dextrose and alcohol on the alveolar carbon dioxide during rest, work and recovery

SUBJECT	TESTS		CARBON DIOXIDE (PER CENT)					
	Type	Number	Rest	SD.	Work	SD.	Recovery	SD.
RCG	C.	1	5.43		5.23		5.29	
	D.	3	5.76	0.083	5.28	0.044	5.28	0.104
	D. + A.	3	5.71	0.118	5.54	0.063	5.30	0.121
CAV	C.	2	6.49	0.200	6.17	0.030	6.43	0.030
	D.	3	6.72	0.081	6.21	0.351	6.21	0.093
	D. + A.	3	6.66	0.067	6.41	0.123	6.33	0.030
DUN	C.	1	6.23		6.06		5.89	
	D.	3	6.51	0.193	6.16	0.090	6.14	0.079
	D. + A.	3	6.48	0.026	6.38	0.186	6.19	0.271

of experiments. However, during the second series of experiments the metal T to which the mouthpiece was attached was provided with a special valve which made it possible to collect alveolar air without the loss of any of the expired air except that which actually entered the collecting tube. The method used was essentially the same as that described by Carpenter and Lee ('33). By means of this device samples of alveolar air were taken at the end of the resting test, about 2 minutes after the end of the work test and again 12 minutes after the end of the work period. The results of these determinations are given in table 3. It will be noted that in all

cases there was a drop in alveolar CO_2 during the work period. In control tests there was usually a tendency for it to return to normal during the recovery period. When dextrose was given there was a slightly greater drop during work and when both dextrose and alcohol were given the drop was smaller and occurred more slowly than in the dextrose tests although it was still somewhat greater than that which occurred in control tests. The resting level of alveolar CO_2 was highest with all subjects after the ingestion of dextrose and lowest in control tests. The values obtained following the administration of dextrose plus alcohol were only slightly lower than those obtained with dextrose alone and the difference is probably not significant. However, in consideration of the reliability of respiratory quotients the most important factor is the change which occurs during the work period. If the drop which occurs following the ingestion of dextrose plus alcohol is significantly less than the corresponding change when dextrose is given alone, this would mean that less CO_2 was washed out of the blood in the dextrose plus alcohol than in the dextrose tests and therefore the lowering of the quotient after alcohol would have less significance. As has already been pointed out this is exactly what happens. However the differences are very slight. Rough computations show that the amount of CO_2 washed out during the dextrose tests in excess of the extra amount given off in alcohol tests is approximately 25 cc. This was given off over a period of more than 20 minutes which would mean that the excess amount retained in alcohol tests could not be more than $1\frac{1}{2}$ cc. per minute. Considering the relatively high level of oxygen consumption during the work period this amount of CO_2 would make a negligible difference in the respiratory quotient. We have calculated that this difference could not be greater than 0.002 and we therefore conclude that in so far as the alveolar CO_2 affects the quotient, differences greater than 0.005 between dextrose tests and dextrose plus alcohol tests are significant. Differences of the same magnitude between control and alcohol tests of the first series of experiments may also be assumed to be significant.

Table 4 shows the average values for the respiratory quotients obtained for rest and work and for the excess metabolism of work plus recovery. In the rest periods there was in all six experiments a significant lowering of the quotient following the administration of alcohol or of dextrose plus alcohol. In the subject RCG, who was used in both series of experiments, it is interesting to note that the lowering of

TABLE 4

The effect of dextrose and alcohol on the respiratory quotient of rest and work and on the excess respiratory quotient of work

SUBJECT	TESTS		REST	SD.	WORK	SD.	EXCESS TOTAL	SD.
	Type	Number						
RCG	C.	5	0.86	0.009	0.95	0.007	1.00	0.013
	A.	5	0.82	0.006	0.92	0.011	0.97	0.017
	D	3	0.87	0.009	0.98	0.004	1.04	0.006
	D. + A.	3	0.79	0.014	0.96	0.007	1.03	0.003
FAH	C.	7	0.80	0.008	0.96	0.015	1.04	0.019
	A.	7	0.76	0.005	0.93	0.012	1.02	0.014
JRM	C.	3	0.81	0.015	0.95	0.025	1.02	0.031
	A.	3	0.80	0.005	0.94	0.012	1.00	0.019
CAV	C.	2	0.84	0.025	0.94	0.001	0.99	0.010
	D	2	0.83	0.013	0.96	0.012	1.02	0.014
	D. + A.	3	0.79	0.012	0.93	0.004	0.99	0.004
DUN	C.	2	0.82	0.029	0.91	0.003	0.96	0.004
	D	3	0.86	0.002	0.95	0.009	1.01	0.018
	D. + A.	4	0.81	0.004	0.92	0.009	0.98	0.009

the quotient was considerably greater when dextrose and alcohol were given together than when alcohol alone was ingested. This is in agreement with the findings of Carpenter and Lee ('37). The administration of alcohol and dextrose plus alcohol was also followed without exception by a lowering of the quotient of both work and excess metabolism. These differences are of less magnitude than those for rest but we nevertheless believe them to be significant.

By assuming an average figure for protein metabolism (in the first series total urinary nitrogen was determined in all experiments and the protein metabolism calculated) we have calculated the quantity of alcohol oxidized in the various periods of all experiments. For these calculations Rosemann's formula as given by Higgins ('17) was used. The figures obtained in this way are given in table 5. The subject FAH was the only one for whom conclusive figures could be obtained for the utilization of alcohol during reclining tests.

TABLE 5

The utilization of ethyl alcohol during rest and work as calculated from changes in the respiratory quotient[1]

SUBJECT	SUBSTANCE GIVEN	MILLIGRAMS OF ALCOHOL OXIDIZED PER MINUTE			TOTAL EXCESS ALCOHOL USED IN WORK AND RECOVERY IN MILLIGRAMS
		Reclining	Sitting	Working	
RCG	Alcohol	.....[2]	28.3	69.7	515.6
RCG	Dextrose + alcohol	No test	57.3	42.1	153.6
FAH	Alcohol	36.3	60.4	93.3	411.5
JRM	Alcohol	.....[2]	11.9	29.5	441.0
CAV	Dextrose + alcohol	No test	43.7	89.3	673.2
DUN	Dextrose + alcohol	No test	47.0	83.2	571.0

[1] The amount of alcohol oxidized per minute was calculated from the non-protein R.Q.'s and the non-protein oxygen consumption according to the following method: $\frac{\text{Control R.Q.—Alcohol R.Q.}}{\text{Control R.Q. —0.67}} \times$ cubic centimeters of O_2 consumed per minute = the cubic centimeters of O_2 used per minute in the oxidation of alcohol. This value divided by 1.4595 (the number of cubic centimeters of oxygen used in the oxidation of 1 mg. of alcohol), gives the number of milligrams of alcohol oxidized per minute.

[2] Data inconclusive.

This subject used about 66% more alcohol while sitting than while reclining. In five of the six experiments there was a marked increase in the utilization of alcohol in the working period over the amount used while sitting at rest. The averages for all six experiments are: sitting, 41.4 mg. per minute and working, 67.8 mg. per minute. This is an increase of a little more than 63%. The average total amount of alcohol used in the excess metabolism of work and recovery for all six experiments was 461 mg. The smallest value obtained was

153.6 mg. for the subject RCG when dextrose plus alcohol was given. This is less than a third of the amount of alcohol used by this subject when alcohol alone was ingested. This would seem to indicate that when there is an abundant amount of carbohydrate present the muscles use this in preference to alcohol. However, this conclusion is not borne out by the results obtained with CAV and DUN. These two subjects who received dextrose plus alcohol showed the greatest amount of alcohol used in the excess metabolism of work and recovery of any of the subjects. We are, therefore, not prepared at this time to make any conclusion as to what effect the simultaneous ingestion of dextrose and alcohol has on the utilization of alcohol during work. We do believe, however, that our experiments indicate that the energy of ethyl alcohol, whether given with dextrose or not, is available for use by the muscles in the performing of work.

The validity of the calculations by means of which the values given in table 5 have been derived rests upon two assumptions. First, that the observed differences between the quotients of control and alcohol tests are of sufficient magnitude to be statistically significant, and second, that these differences are the result of changes in the character of the fuel being oxidized by the various subjects, that is, the substitution of alcohol for carbohydrate and fat. In order to test the statistical significance of the results the probable errors of the differences between the respiratory quotients of the excess metabolism of work in control and alcohol tests have been calculated and the ratios between the observed differences and these probable errors determined. In the first series of experiments the observed difference was 2.1 times the probable error for the subject RCG, 1.25 times the P.E. for FAH and only 0.83 times the P.E. for JRM. In the second series of experiments the difference is twice the probable error for RCG, 3.0 times the probable error for CAV and 2.5 times the probable error for DUN. Now it must be remembered that a difference so slight as to be without significance in a single series of experiments becomes significant if it is shown that differences of this magnitude or

greater and in the same direction, invariably occur in similar series of experiments. Thus, if the changes of quotient were fortuitous in these experiments there would be one chance in two trials that the quotient would be lower in alcohol than in control tests, but that the quotients would be lower in the alcohol than in the control test for each of six different and distinct series of tests the chances are one-half raised to the sixth power or one-sixty-fourth. Thus slight differences such as those for JRM and FAH in the first series of experiments which would be without significance if considered separately become significant when considered in the light of the results obtained with the other subjects. Therefore these results justify the conclusion that there is a true lowering of the respiratory quotient of the excess metabolism of work following the ingestion of ethyl alcohol.

There are, however, many factors aside from the nature of the fuel being oxidized which will materially influence the value of the respiratory quotient. Among these may be mentioned the following: Changes in the ventilation rate, alterations in the acid base equilibrium, circulatory adjustments, and the interconversion of nutrients. It has already been shown by the ventilation equivalents given in table 2 that no changes in ventilation rate occurred with any of our subjects of such a magnitude as to produce variations in the quotients. Furthermore while the changes which occurred in the alveolar carbon dioxide in these experiments indicate that there was a shift in acid base equilibrium probably occasioned by an increase in blood lactic acid, the magnitude of this change was practically identical in control and alcohol tests and therefore without effect on the final results. Furthermore, while it is altogether possible that the quotients were affected by circulatory adjustments between the resting and work periods it seems entirely reasonable to assume that these adjustments were the same in the control as in the alcohol tests and that they also were without effect upon the final results. Finally, while it is true that the drop in the quotients observed may have been due to the interconversion

of the alcohol ingested to some other substance rather than to its direct oxidation, the substance produced from the alcohol would presumably be available for oxidation at some later time and the final results would therefore be the same as though it had been oxidized directly. For these reasons therefore, we feel justified in concluding that the drop in the respiratory quotient of the excess metabolism of work following the ingestion of ethyl alcohol is a valid, though presumptive, measure of the utilization of alcohol by the muscles in the performing of work.

SUMMARY

The effects of the ingestion of small amounts of ethyl alcohol alone and of dextrose plus ethyl alcohol on the respiratory metabolism of five male human subjects have been investigated by means of the Tissot-Haldane technic. The results justify the following conclusions:

1. Alcohol produced no specific dynamic action during the first 2 hours following its ingestion.
2. There was no significant change in the ventilation equivalent or in the alveolar carbon dioxide following the ingestion of alcohol.
3. Utilization of alcohol while resting was greater when given with dextrose than when given alone.
4. The rate of oxidation of alcohol in the body was higher in the sitting position than when reclining and still higher when working on the bicycle ergometer than when sitting at rest. The results are interpreted as presumptive evidence that the muscles are capable of using the energy of ethyl alcohol in the performing of work.

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RECOVERY FOLLOWING SUPPRESSION OF GROWTH IN THE RAT [1, 2]

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ONE FIGURE

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The question of the influence of the length of the period of inanition upon the ability to resume growth after optimal nutrition has again been provided, is one which has long been discussed. The impossibility to completely suppress the growth impulse in rats, even by long-continued restriction of growth, has been stressed by Osborne and Mendel ('14, '15, '16), by Jackson ('25, '36) and by McCay, Crowell and Maynard ('35). On the other hand, Stewart ('16) and Jackson and Stewart ('20) found that there was a permanent stunting effect in rats underfed from weaning, the recovery upon refeeding being least complete in those animals whose growth was previously retarded for the longest time.

In the present study emphasis has been placed on the influence of the length of time of growth restriction prior to realimentation upon the subsequent development of the experimental animals. Two types of dietary procedures were used to retard the growth of young albino rats, namely, the restriction of a) inorganic salts and b) energy intake. Male rats of 100 gm. body weight (35 days old, approximately) were

[1] Some of the data in this paper are taken from a dissertation presented by Miriam F. Clarke in partial fulfillment of the degree of doctor of philosophy, Yale University, 1933.

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fed the low-salt diet for 3, 6 or 12 weeks during the period of restriction of growth. Controls of the same age were given either the adequate synthetic diet for similar experimental periods (age controls) or a complete synthetic diet limited in caloric intake to that of the low-salt rats whose food consumption was voluntarily reduced about 50% (calorie controls). It was thus possible to differentiate between the effects of the reduction of mineral salts and the limitation of the sources of energy. At the end of the period of the restriction of growth, both the low-salt rats and their calorie controls were realimented with an adequate synthetic diet fed ad libitum, the period of refeeding varying from 6 to 12 weeks as indicated by the tables and figure.

Besides weekly weights of the rats, the following values were recorded when each rat was killed: nose-anus length, weight of the kidneys, spleen, testes, femurs and incisors. Weights and other data concerning the bones and teeth have already been published (Clarke and Smith, '35; Clarke, Bassin and Smith, '36).

RESULTS

Weight and length

The retardation or cessation of growth was followed promptly by very rapid growth when the diet was made adequate (fig. 1). The curves show a striking difference between the recovery which followed a short period of suppression and that following longer periods. After 3 weeks stunting there was over-compensation both in the realimented low-salt rats and in the realimented calorie controls. Following restriction for 6 and 12 weeks, on the other hand, there was an apparently permanent stunting effect, especially noticeable in the low-salt rats. That the 'critical time' insofar as the completeness of recovery is concerned, lies between 3 and 6 weeks is shown by the curves c and d in figure 1. The rats represented by curve c, on the inadequate low-salt diet for 3 weeks, more than recovered weight lost through the restriction of mineral salts in the diet, indicated by the cross-

ing of the age control curve a. The same phenomenon of overcompensation is true of rats (curve b) restricted only in respect to the amount of energy consumed (calorie controls). The curve d represents the growth of animals in which the deleterious effects of 6 weeks on the mineral-restricted diet can be observed.

The experiments were not continued sufficiently long to determine whether the losses incurred by limiting the growth could ultimately be regained. During the last 4 weeks of the growth which followed the 6 weeks of restriction, the

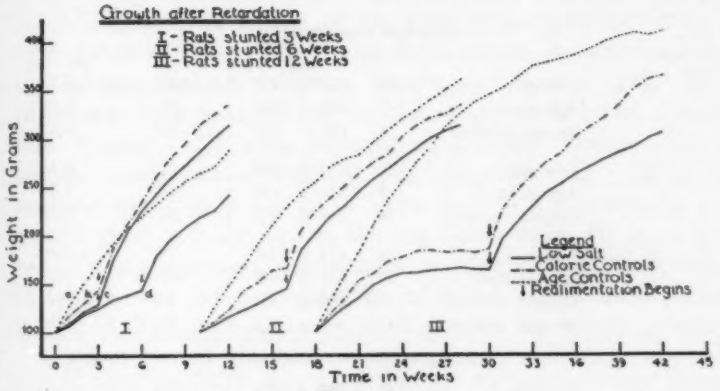

Figure 1

animals in all three groups (curve II, fig. 1) were growing at approximately the same rate, 1 gm. per day. At the end of the experiment the low-salt rats were 13% below the age controls in weight, while the calorie controls were only 7% below. Following the longer (12 weeks) period of limited growth, the initial acceleration upon realimentation was almost as striking and growth at a fairly rapid rate was sustained throughout the experiment (curve III, fig. 1). The rate of growth during the last 4 weeks (twentieth to twenty-fourth week) was approximately 0.5 gm. per day for the age controls, 1 gm. for the realimented low-salt rats, and 1.5 gm. for the realimented calorie controls. The possibility of

ultimately completely regaining body weight has therefore not been ruled out.

During the period of stationary weight the increase in length of the low-salt rats had practically ceased, while the calorie controls gained about half the amount gained by the age controls (table 1). The average length in millimeters

TABLE 1

Length of body (nose-anus)

WEEKS ON EXPERIMENT	NUMBER OF RATS IN GROUP		MEAN LENGTH	LENGTH COMPARED WITH AGE CONTROLS	WEIGHT / LENGTH RATIO
Low-salt experiment					
			mm.	%	gm./mm.
3	Low-salt	10	*176* ± 4.5[1]	91	0.73
	Age control	7	193 ± 3.3	100	0.92
	Calorie control	4	181 ± 4.3	94	0.80
6	Low-salt	11	*183* ± 5.9	87	0.81
	Age control	7	210 ± 4.9	100	1.12
	Calorie control	4	199 ± 3.8	95	0.93
12	Low-salt	18	*175* ± 8.0	76	0.89
	Age control	10	230 ± 8.6	100	1.30
	Calorie control	9	*202* ± 4.8	88	0.91
	Weight control[2]	10	*180* ± 2.2	78	0.87
Realimentation experiment					
3 + 9[3]	Low-salt	10	227 ± 11.6	103	1.38
	Age control	8	221 ± 10.8	100	1.31
	Calorie control	4	*236* ± 5.8	107	1.43
6 + 6	Low-salt	11	*210* ± 8.2	95	1.15
	Age control	8	221 ± 10.8	100	1.31
6 + 12	Low-salt	10	*225* ± 9.2	94	1.39
	Age control	7	240 ± 8.3	100	1.49
	Calorie control	4	*233* ± 3.0	97	1.44
12 + 12	Low-salt	19	*213* ± 8.6	86	1.44
	Age control	9	249 ± 4.9	100	1.65
	Calorie control	4	*233* ± 8.9	94	1.56

[1] Standard deviation.

[2] Young rats, having same body weight as low-salt rats after 12 weeks.

[3] Number following plus mark indicates number of weeks of realimentation.

Values in italics represent cases where the experimental mean is statistically different from the mean of the corresponding age controls.

of the low-salt rats at 12 weeks was actually less than that of the weight controls, animals of the same body weight but about 10 weeks younger. This apparent shrinkage may be due to a slight dorsal curvature characteristic of rats kept on the low-salt diet for extended periods. Arching of the back has been described for rats subjected to inanition by different methods (Aron, '14; Jackson, '15; Winters, Smith and Mendel, '27).

The response of the axial skeleton to adverse dietary conditions can be contrasted with that of the appendicular skeleton. In the former, growth ceased (table 1) while in the latter increase in length of the long bones proceeded at a rate about half the control rate both on the low-salt and the low-calorie regimes (Clarke, Bassin and Smith, '36). This difference was brought out by the study of Winters, Smith and Mendel ('27) with younger rats under similar experimental conditions in which it was observed that "while the animals made only 8.7 to 19.7 per cent of the normally expected gain in body length, the leg bones made 36 to 41 per cent." In the present experiment, with older rats, the growth in length was more completely inhibited while the increase in size of the long bones continued as with the young animals.

Comparison of the body length of experimental (low-salt and calorie control) rats with that of the age controls (table 1) shows that growth in length, considerably slowed down during the restricted period, accelerated rapidly during realimentation. In length as well as in weight the $3 + 9$ group over-compensated. The high weight/length ratios for this group, however, indicate that recovery was less prompt than was that in body weight; this may arise from the fact that growth in length was affected relatively less than body weight during the period when development was checked. Ratios for rats stunted for longer periods before realimentation, show weight/length values lower than those of the age controls, indicating that the growth was distorted by a persistent increase in length when recovery of body weight was far from complete. Inorganic salts as the limiting factor had a

greater influence upon the subsequent growth in length than the mere reduction of the energy intake. This is understandable when one considers the pathology of the bones already described for rats under these experimental conditions (Arnim, Clarke, Anderson and Smith, '36), and the dependence of the length upon the growth of the skull and vertebral column. The response to realimentation of the axial skeleton, represented by body length, is similar to that of the long bones as representatives of the appendicular skeleton. Although affected unequally by faulty diets, they both responded by over-compensation after the shortest period of retardation, and by incomplete recovery after the longer periods.

The end of the experiment which was continued for the longest time found the realimented low-salt rats only 14% below the age controls in length whereas they were 25% below in weight. Moreover the linear growth was continuing at a fairly uniform rate, whereas the weekly weight increments were decreasing. Thus a persistent distortion of body proportions was brought about by the conditions of the experiment. That the phenomenon of growth in length may be less permanently impaired than growth in weight is more comprehensible when one considers the fact, pointed out by Outhouse and Mendel ('33), that the normal curve of linear growth tends to flatten at an earlier age than the curve for growth in body weight. At 100 gm., the starting weight of the present experiment, the normal male rats had attained about 70% of their final length, whereas they weighed at that time only 25% of their final weight.

A comparison of the weight/length ratios at the end of realimentation of the groups which had been restricted for 3 weeks with those restricted for 6 or for 12 weeks, emphasizes the fact that the distortion of development at the end of the experiment is definitely correlated with the length of time during which the growth was initially retarded.

Organ weights

The growth of the kidneys and the testes persisted during the salt-restricted period, resulting in organs which bear a distorted ratio to body weight (table 2). The spleen, on the other hand, atrophied during the period when salts were withheld (see also Swanson, Storvick and Smith, '36), a phenomenon which occurred also in the calorie-restricted rats, and appeared therefore to be associated with inanition. The kidneys tended to become hydrated (Swanson and Smith, '36) while the moisture of the spleen remained like that of the controls. The recovery during realimentation is indicated by increase of absolute weights of kidneys, testes and spleen, as well as by the return to nearly normal organ/body weight ratios. The average weights of these organs in the 3 + 9 groups (low-salt and calorie control) often exceeded by a small amount the corresponding value for the age controls. This is to be expected inasmuch as these organ weights bear a close relationship to body size (Donaldson, '24) and all are known to recover after inanition (Jackson, '25).

DISCUSSION

The rats in the present study may be divided into two categories, those which over-compensated after being restricted in growth and those which failed to overtake their unstunted controls. In each case the kidneys and testes as well as incisors (Clarke and Smith, '35), and femurs (Clarke, Bassin and Smith, '36) continued to grow to such an extent that they were large in proportion to body size at the end of the stunting period. The kidneys and testes as well as the spleen, returned promptly to normal size with respect to body weight when realimentation began. The difference between experimental (both low-salt and calorie control) rats and their age controls appeared to be, to a considerable extent, one of body fat, a difference that was soon made up when growth was allowed.

Whether the animals over-compensated in growth or failed to equal their controls in weight seems to be correlated with

TABLE 2

Weight of kidneys (2), testes (2) and spleen

WEEKS ON EXPERIMENT	GROUP	KIDNEY WEIGHT	KIDNEY / BODY WEIGHT × 100	KIDNEY MOISTURE	TESTES WEIGHT	TESTES / BODY WEIGHT × 100	SPLEEN WEIGHT	SPLEEN / BODY WEIGHT × 100	SPLEEN MOISTURE
Low-salt experiment									
		gm.		%	*gm.*		*gm.*		%
3	Low-salt	1.256	1.09	75.9	2.040	1.6	0.301	0.23	78.0
	Age control	1.315	0.73	75.3	2.218	1.3	0.541	0.31	78.0
	Calorie control	1.080	0.75	75.6	1.941	1.4	0.389	0.27	77.9
6	Low-salt	1.309	0.88	76.4	2.210	1.5	0.288	0.19	77.9
	Age control	1.525	0.64	75.6	2.642	1.1	0.415	0.18	77.8
	Calorie control	1.196	0.65	76.2	2.373	1.3	0.407	0.22	77.5
12	Low-salt	1.295	0.85	76.9	2.249	1.3	0.313	0.21	77.3
	Age control	1.898	0.58	76.3	2.819	0.9	0.542	0.17	77.4
	Calorie control	1.214	0.66	75.8	2.469	1.3	0.312	0.17	77.2
	Weight control	1.145	0.73	76.4	1.885	1.2	0.487	0.31	78.0
Realimentation experiment									
3 + 9	Low-salt	1.704	0.56	76.2	2.879	0.9	0.536	0.17	77.5
	Age control	1.712	0.59	76.1	2.823	1.0	0.500	0.17	77.5
	Calorie control	1.873	0.56	75.7	2.679	0.8	0.512	0.15	77.3
6 + 6	Low-salt	1.519	0.64	76.4	2.598	1.1	0.443	0.18	77.6
	Age control	1.712	0.59	76.1	2.823	1.0	0.500	0.17	77.5
6 + 12	Low-salt	1.758	0.56	77.0	3.062	1.0	0.431	0.14	77.2
	Age control	2.026	0.57	76.4	3.172	0.9	0.473	0.13	77.2
	Calorie control	1.970	0.59	75.5	3.147	1.0	0.474	0.14	77.1
12 + 12	Low-salt	1.718	0.56	76.5	3.031	1.0	0.543	0.18	77.5
	Age control	2.224	0.54	76.7	3.300	0.8	0.792	0.19	77.2
	Calorie control	1.986	0.55	76.1	3.192	0.8	0.579	0.16	77.3

skeletal size. Although the skeleton is less readily influenced than other parts by nutritionally inadequate diets, and has often been known to persist in growth when the growth of the animal as a whole has ceased (Aron, '11, '14; Waters, '08), once it has become damaged, its subsequent growth and that of the body as a whole are seriously interfered with. Comparing the total growth with that of the skeleton as exemplified by the dimensions of the femur, fibula, radius and humerus (Clarke, Bassin and Smith, '36), it becomes apparent that the general growth follows closely the development of the skeleton. When the dimensions of these bones of the experimental rats exceeded those of the age controls in this study, the body weight also exceeded the control weight. This was true in spite of the fact that the bones of the low-salt rats actually weighed less than corresponding bones of age controls. Likewise experimental reduction in the size of the bones was associated with permanent dwarfing of the body as a whole. That the growth of the body depends on the skeletal pattern has been demonstrated by the experiments described by Jackson ('36) in which male rats failed to recover weight lost during 15 weeks of suppression of growth due to protein insufficiency. Here incomplete recovery in weight was associated with reduced skeletal size, exemplified by the nose-anus length, the weight of the skeleton plus musculature and of the humerus and femur. The females on the other hand, exhibited complete recovery with respect to both the total body growth and the skeletal growth.

The experiments described herein emphasize the fact that the sequel to suppressed growth insofar as recovery is concerned, depends on the length of time during which the growth was inhibited as well as upon the nature of the deficiency. Short periods of inhibition act as a stimulus for growth. This phenomenon of acceleration may be similar to that observed by Stearns and Moore ('31) in a child 3½ years of age recovering from severe malnutrition due to multiple causes. Increase in weight of this child during the 9 months he was under observation was about nine times the normal increase for

that age, while growth in length exceeded the normal rate about fourfold. Thus they conclude that "if ever a child exerts his maximum capacity for growth, it is during rapid recovery from severe malnutrition."

The parallelism which exists between the skeletal pattern and the size of normal rats, growing at different rates, has been emphasized by Outhouse and Mendel ('33). Likewise, in experiments involving prolonged periods of stationary weight (McCay, Crowell and Maynard, '35), the "bone measurements fit the general picture of the final size attained. . . ." The early studies of Osborne and Mendel ('14, '15) contain no measurements of the skeleton, so that it would be impossible to determine from their data whether the complete recovery of body weight exhibited even after prolonged inanition was accompanied by complete return to normal skeletal magnitude. The tendency for the proportions of the body to remain in harmony despite adverse conditions is very great. Jackson and Stewart ('20) observed that the bodies of rats underfed from birth for long periods, then refed to maximum body weight, had nearly normal proportions, though they were permanently dwarfed. Whether the diet is lacking in the materials for skeletal growth, as in the low-salt diet, or only in total energy available, if it is fed sufficiently long the growth of the skeleton can be seriously interfered with. Both acceleration and retardation of skeletal development are reflected in somatic growth.

SUMMARY

The development of young rats following the suppression of growth due to restriction of mineral salts or energy intake was studied. The criteria used were increase in weight and length of the body and the weight of the kidneys, testes, spleen, femurs and incisors. The animals given the restricted diets for the shortest period, 3 weeks, over-compensated by exceeding their controls at the end of 9 weeks of refeeding. Rats stunted by either method for longer periods failed within the period of observation to regain losses suffered during

suppressed growth, although those restricted in energy intake alone made much greater gains in the time allotted and gave indications that complete recovery could ultimately occur. The weight of organs studied resumed a relationship to body weight like that of controls. Whether the stunted animals overtook and exceeded their controls in weight or failed to do so depended on the length of time their growth was suppressed which in turn influenced the growth capacity of the skeleton, both axial and appendicular.

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THE RELATIVE EFFECTS OF CERTAIN SACCHARIDES AND OF VITAMIN D ON MINERAL METABOLISM OF RATS [1]

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THREE FIGURES

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In a previous publication from this laboratory (Outhouse, Smith, Merritt and White, '37) the feeding of lactose was reported to have an accelerating effect on the calcification of bone which was not participated in by certain other naturally occurring carbohydrates. These results were in confirmation of those published earlier by Jarvis ('30), Kline, Keenan, Elvehjem and Hart ('32), and Whittier, Cary and Ellis ('35). In view of these findings, it was considered that studies involving the metabolism of the bone-building elements might aid in explaining the manner in which this calcifying action is brought about. The present paper deals with studies designed to show the influence of this carbohydrate on calcium, phosphorous, and magnesium metabolism. The effects were compared with those obtained by the use of two other commonly used carbohydrates (starch and sucrose) and the calcifying agent par excellence, vitamin D. In general, the data indicate that both lactose and vitamin D acted similarly in that they stimulated the retention of all three elements in excess of that induced by starch or sucrose, but differed primarily in that more of calcium and of phosphorous was stored under the influence of vitamin D.

[1] A portion of these data was presented before the American Chemical Society, Cleveland, 1934.

EXPERIMENTAL

The procedures used in this study were those described for series 2 in the aforementioned paper from this laboratory. In fact, the bone-ash values of the twelve groups of rats herein reported are presented in table 4 of that paper (groups 4203 to 4738 inclusive). It should be reiterated that control-feeding technic was adapted to groups of four litter-mate rats, each of the four animals receiving a different ration but an equivalent number of calories and practically the same quantity of mineral salts. The rats were maintained on the rations for 28 days and were 56 days of age when the experiment was terminated.

Between the twelfth and twenty-fourth days of the experiment the excreta were collected for analysis. Ferric oxide was fed as a feces marker. During this 12-day period the rats were placed in cylindrical Pyrex glass cages of the type described by Smith and Brooke ('30–'31). Both urine and feces fell upon filter paper (Whatman no. 40) which had been treated according to the method of Brooke and Smith ('33) with 2 N. acetic acid and thymol. The feces were collected daily and at 4-day intervals the cages were carefully washed with redistilled water. The combined washings, together with the filter paper, were digested on a steam bath, filtered, and washed with redistilled water until there was no trace of residual urine. This filtrate was then evaporated to a convenient volume. Urine and feces were digested separately in dilute acid according to the method of Stearns ('28–'29). Both digests were made to volume, and aliquots were used for analysis.

Calcium and magnesium were determined on the excreta by the procedure of Morris, Nelson and Palmer ('31) and on the rations by the gravimetric method of McCrudden ('11–'12). In the determination of phosphorus the method of Meigs, Blatherwick and Cary ('19) was used for the rations and that of Fiske and Subbarow ('25) for the excreta.

RESULTS

Bone-ash values. The bone-ash values are given at the top of the columns in figures 1 to 3. In each of the twelve groups, the cod liver oil ration and the lactose ration induced the deposition of more ash than did the starch or the sucrose diets of the litter mates. A comparison of the lactose and cod liver oil rations showed that in seven of the twelve groups the animal on the latter ration had more ash in its bones than had its litter mate. For this comparison (Student, '15–'17) the statistical odds of 27 to 1 were too low to be more than suggestive that cod liver oil, at the levels fed, was the better calcifying agent.

Weight of feces. The fecal material appeared to be bulkier in the case of the lactose-fed rats. Upon removal of the moisture in vacuo, the dry weight of the stools was found to be greatest for the rats receiving lactose in each of the ten litter-mate groups and least for those receiving cod liver oil in nine of the ten groups. Statistically, these differences were significant. No appreciable disparity could be found between the weight of feces of litter mates on the other two rations. The greater weight of the stools of the lactose-fed animals could not be explained on the basis of excessive loss of calcium or phosphorus whereas the low values found for the cod liver oil animals were paralleled by a decreased excretion of these two elements. A study was not made of the bacterial content of the stools as was done by Mitchell ('26–'27 a, b).

CALCIUM METABOLISM

Calcium intake. The data for the calcium metabolism of the individual animals are presented graphically in figure 1. The calcium content of the rations approximated 0.50%. Considerable variation occurred in the amount ingested by the twelve different groups. For the 12-day period the intake ranged from 261 to 500 mg.

Calcium retention. For the different rations, characteristic variations in the retention of calcium were found. The rats fed either lactose or cod liver oil retained more calcium than

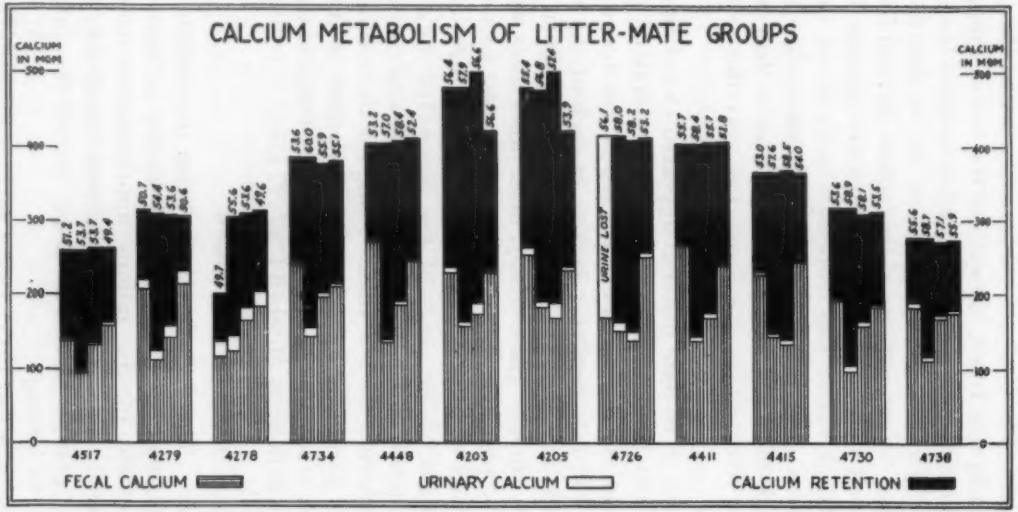

Fig. 1 The slight inequality of amounts of calcium consumed by the litter mates (as noted particularly for groups 4203 and 4205) was due to small differences in the mineral content of the rations and not to a disparity in the quantity of food eaten.

In group 4278 the rat on the starch ration developed anorexia during the latter part of the metabolism period. Therefore the food of the other three animals was equalized with that of their litter mates in group 4279.

did their litter mates on either the sucrose or the starch rations in each of the twelve groups. On comparing these two calcifying agents, however, the latter was found to favor greater retentions in all but three of the groups. Statistical odds of 293 to 1 indicate a high degree of probability that the addition of vitamin D stimulated the greater storage of calcium. No difference could be found between the retention by litter mates on the starch and sucrose rations. For the rats on a given ration the retention of this element was definitely related to intake as indicated by high coefficients of correlation [2] between these two factors.

The amount of calcium retained in relation to that ingested was fairly consistent for the rats on a given ration. The average values for the starch, the cod liver oil, the lactose, and sucrose rations were 37 $\pm$ P.E. 4.5, 61 $\pm$ P.E. 3.0, 51 $\pm$ P.E. 5.6, and 38 $\pm$ P.E. 4.2% of the intake respectively. Statistically significant differences existed between these values for all the rations except between the values for the starch and sucrose rations. These data indicate that, in stimulating calcium storage, the lactose ration was 38%, and the cod liver oil ration 62% more effective than the other two rations.

Urinary calcium. The quantity of calcium excreted by way of the kidney was very slight and bore no relationship to the total amount eaten or excreted. However, it was influenced by the type of ration eaten. The ingestion of lactose and of cod liver oil was accompanied by a greater loss of calcium in the urine than was the ingestion of sucrose. A similar relationship occurred in a comparison between the values of the lactose and starch rations but not between those for the cod liver oil and starch rations.

Fecal calcium. The differences between the effects of the four rations on the amount of calcium lost in the feces showed the same relationships as was found in their effects on total calcium retention. Thus, on both the lactose and the cod liver oil rations, litter mates excreted less fecal calcium than those

[2] Correlation coefficients were considered to be significant if the values were four times as great as the probable error.

on the other two rations in 100% of the cases. In comparing the two calcifying agents the statistical odds were 521 to 1 that less calcium was excreted by the animals receiving cod liver oil. For any one ration the fecal calcium values tended to be related to the quantity of calcium eaten, but the correlation coefficients were significant only for the starch ration and for the cod liver oil ration.

PHOSPHORUS METABOLISM

Phosphorus intake. The data for phosphorus metabolism are given in figure 2. The phosphorus content of the ration approximated 0.63% and the quantity consumed during the 12-day period varied, between the twelve groups, from 196 to 580 mg.

Phosphorus retention. Cod liver oil ingestion was associated consistently with a greater retention of phosphorus than was found on the other three rations. (In one litter-mate group the lactose-fed rat stored more.) On the other hand, the lactose-fed rats had a tendency to store more of this element than did their litter mates on the starch and sucrose rations. Statistical odds of 36 to 1 and 525 to 1, respectively for these last two comparisons were high enough only in the latter case to give practical certainty that a true difference existed in the amount of phosphorus stored.

The retentions of this element by the animals on a given ration tended to be dependent on the quantity of phosphorus consumed. High correlation coefficients between these variables were obtained for all the rations. The retentions, in terms of per cent of intake, gave average values of 17 $\pm$ P.E. 4.5, 27 $\pm$ P.E. 3.5, 22 $\pm$ P.E. 4.0, and 18 $\pm$ P.E. 3.5 for the starch, the cod liver oil, the lactose, and the sucrose rations respectively. Significant differences between these values were found only for the cod liver oil ration in relation to the other three diets.

Urinary phosphorus. For urinary phosphorus the only significant difference between the animals of a quartet was found in the case of the cod liver oil-fed rats. These showed greater

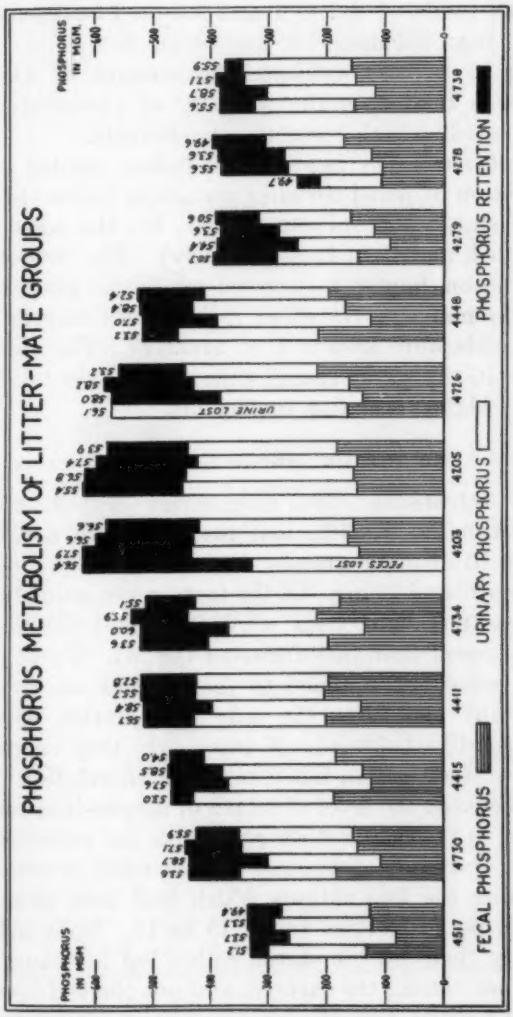

Fig. 2 The slight inequality in the amounts of the phosphorous consumed by the litter mates (as noted particularly for groups 4203 and 4205) was due to small differences in the mineral content of the rations in the quantity of food eaten.

In group 4278 the rat on the starch ration developed anorexia during the latter part of the metabolism period. Therefore the food of the other three animals was equalized with that of their litter mates in group 4279.

excretions than did the litter mates fed either the starch or sucrose rations (odds of 132 to 1 and 908 to 1 respectively) but no greater than did those fed the lactose diet.

In contrast to urinary calcium, the amount of urinary phosphorus, was related to the quantity of phosphorus ingested as indicated by high correlation coefficients.

Fecal phosphorus. The ingestion of lactose tended to reduce the excretion of phosphorus in the stools below that obtained on the starch and sucrose rations, but the odds were very low (36 to 1, and 49 to 1, respectively). The rats on the cod liver oil ration, however, excreted much less phosphorus than their litter mates on the other rations with only two exceptions (the odds were 4999 to 1, or greater). The quantity of phosphorus in the stools varied with phosphorus intake as shown by very high correlation coefficients.

MAGNESIUM METABOLISM

Magnesium retentions. The magnesium content of the rations approximated 0.083%, and the quantities consumed by the twelve litter-mate groups during the 12-day period ranged between 42 and 73 mg. Of the forty-seven animals (for which complete metabolic data were available) twelve lost more of this element than they ingested (fig. 3). These negative balances were not confined to the animals on any one ration or to any one litter (in only one quartet were all members in negative balance). Neither were they related to the quantity of food eaten, inasmuch as a correlation could not be found between the level of intake of magnesium and the amount stored in the body. With respect to the retentions of this element a statistical difference did not exist between the pairs consuming the two rations which had been shown to possess calcifying properties (odds 15 to 1). Both induced greater storage than did the starch ration but in comparison with the sucrose ration, the lactose, and not the cod liver oil ration, caused a slight, though significant, increase in storage.

Urinary magnesium. The lactose ration stimulated the excretion of a significantly greater quantity of magnesium

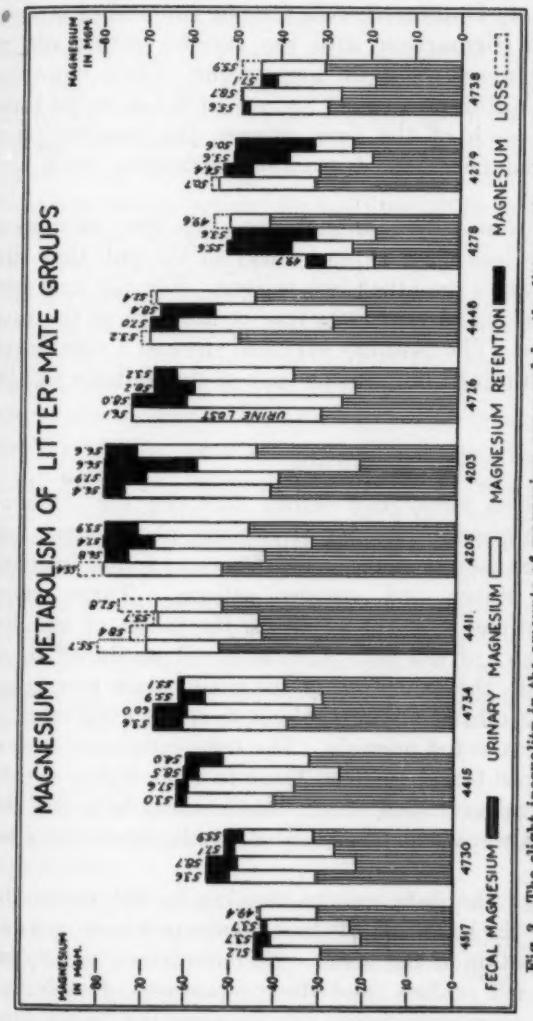

Fig. 3 The slight inequality in the amounts of magnesium consumed by the litter mates (as noted particularly for groups 4203 and 4205) was due to small differences in the mineral content of the rations and not to a disparity in the quantity of food eaten.

In group 4278 the rat on the starch ration developed anorexia during the latter part of the metabolism period. Therefore the food of the other three animals was equalized with that of their litter mates in group 4279.

in the urine than did the starch or sucrose ration. The ration containing vitamin D differed from the one containing lactose in that only in comparison with the sucrose ration did a greater loss of magnesium in the urine occur. These were the only significant differences found for any of the possible comparisons. For each of the four groups, the quantity lost through this channel showed a close correlation with the amount ingested.

Fecal magnesium. The animals on the two calcifying rations excreted less magnesium by way of the gut, than did their litter mates on the other two rations. For the first two rations, the fecal magnesium was less in the case of the rats receiving lactose. The quantity excreted showed a significant correlation with intake only in the case of the animals on the starch ration.

DISCUSSION

This metabolism study has shown that the ingestion of lactose caused a greater quantity of calcium, phosphorus, and magnesium to be stored in the body than was found in litter mates on the starch and sucrose rations. These more favorable retentions were, in all cases, the result of smaller excretions by way of the gut. Cod liver oil produced these same effects with the exception of the magnesium retentions of the cod liver oil-fed animals which were no greater than those of the sucrose-fed animals. The only consistent difference that could be found between these two calcifying agents was that the fecal excretion of all elements was less, and the total retentions were greater in the animals receiving vitamin D.

An analysis of the data was undertaken to determine the extent to which the retention of these elements was concerned with the calcification of the bones. No correlation was found between bone-ash values and the retention of calcium, phosphorus or magnesium when the data for the animals on any one ration were considered. However, for a litter-mate group, within which variations due to food intake and litter

differences were eliminated, a definite relationship existed for calcium and for phosphorus. Of the fifty-six possible comparisons [3] (i.e., starch vs. cod liver, starch vs. lactose, lactose vs. cod liver, lactose vs. sucrose, and sucrose vs. cod liver oil) there were fifty-three that showed not only higher bone-ash values but also a greater retention of calcium. Greater phosphorus retentions were also associated with the higher bone-ash values induced by cod liver oil in twenty-eight of the twenty-nine instances. On the other hand, in only fifteen of the twenty-two instances (69%) in which the ingestion of lactose was attended by a higher ash content of the bone was there a parallelism in phosphorus retentions. In respect to magnesium, the relationship held for cod liver oil in only twenty-one out of thirty instances and for lactose in only seventeen of twenty-six instances (i.e., 70 and 65% respectively). From these data it would appear that the calcification, which was induced by vitamin D, was the result of a stimulation to the storage of both calcium and phosphorus, whereas that attending the ingestion of lactose was due primarily to calcium. The inconsistent parallelism between magnesium retentions and bone-ash values indicates the possibility that a part, at least, of the excess magnesium retained by the animals receiving either of the calcifying agents over that retained by the animals on the other rations may have been stored in some tissue other than bone.

SUMMARY

1. The influence of starch, lactose, sucrose, or cod liver oil rations on calcium, phosphorus and magnesium metabolism was studied.

2. Control-feeding technic was adapted to groups of four litter-mate rats, each of which received one of the above rations.

3. The lactose and cod liver oil rations caused greater retentions of all three elements than did the starch or sucrose rations with one exception, i.e., the cod liver oil ration caused no greater magnesium storage than did the sucrose ration.

[3] There were fewer than the sixty possible comparisons due to the loss of part of the metabolic material.

4. Greater increments in retentions were obtained in the cod liver oil-fed animals than in the lactose-fed rats.

5. The higher bone-ash values resulting from the feeding of vitamin D were accompanied by greater retentions of calcium and phosphorus. Similar results were found for the lactose animals except in the case of phosphorus, for which only 69% of the instances showed a parallelism to bone ash.

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THE USE OF FIBRIN IN SYNTHETIC DIETS

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ONE FIGURE

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Since the so-called synthetic or purified diets have been employed for experimental studies casein has been the most commonly used protein. Several factors have led to this choice, viz., a) the facility with which casein can be obtained in a relatively pure state, b) its comparatively low cost, c) the readiness with which it can be extracted with various solvents for the purpose of purification, d) the ease with which it can be ground and incorporated into the ration, e) its high biological value and finally f) its freedom from any toxic principle even when fed at high levels. There are times, however, when casein is not entirely satisfactory and it is desirable to use some other protein. For example, casein is a conjugated protein containing phosphoric acid and consequently cannot be used in low phosphorus diets. Also casein as usually obtained in the crude state contains many impurities of nutritional significance which would be expected as its original source is milk. Although, as previously stated, casein can be easily extracted with a wide variety of solvents, it is not a simple problem to remove all of these impurities. Day and Darby ('37) have recently emphasized the difficulties of purifying casein sufficiently for use in the production of cataracts in rats resulting from a deficiency of riboflavin. They have pointed out that some samples of commercially purified 'vitamin-free' casein are unsatisfactory for this purpose. In

this and similar cases it would be well to have another protein to use instead of casein or at least to compare with casein when the latter is suspected of giving undesirable results. There are probably numerous other instances in which the use of some protein other than casein would be advantageous providing it also had most of the desirable properties of casein listed above.

Our interest in this problem arose during an attempt to prepare a diet very low in phosphorus. After considering the various proteins available, blood fibrin was chosen as being most likely to fulfill the larger part of the desired qualifications. Beef-blood fibrin is now available in any quantity and at a price comparable with that of casein, and the following experiments show that it can be used readily as the source of protein in various synthetic rations.

EXPERIMENTAL

Beef-blood fibrin is easily ground to any desired degree of fineness and can be extracted with alcohol or ether without difficulty. With water it swells more than casein and tends to form a jell. In the crude, air-dried state it contains approximately 13.3% nitrogen, 5.0% alcohol-ether soluble extract, 0.08% calcium and 0.10% phosphorus. After extracting with alcohol and ether the nitrogen is increased to 14.3%, the phosphorus reduced to 0.085% and the calcium remains unchanged. Very little material is extracted by ether following hot-alcohol extraction in a Soxhlet apparatus.

As a preliminary growth experiment the crude product was fed at a level of 18% with a basal diet of dextrinized starch 70, yeast 6, salt no. 40 (Steenbock and Nelson, '23) 4, agar 2 and cod liver oil.[1] On this diet young rats grew at a rapid rate and appeared normal. In subsequent experiments the growth-promoting properties of alcohol-extracted fibrin were compared at different levels with alcohol-extracted casein. The results are given in figure 1. As can be seen the biological

[1] Whenever cod liver oil was included in the diet 0.25 cc. was given directly three times per week.

value of fibrin, as determined by this method is equal to, if not somewhat greater than, casein. When these two proteins were fed at equal-weight levels the fibrin diets contained slightly more nitrogen than the casein diets as the former protein had a little higher content of nitrogen than did the latter.

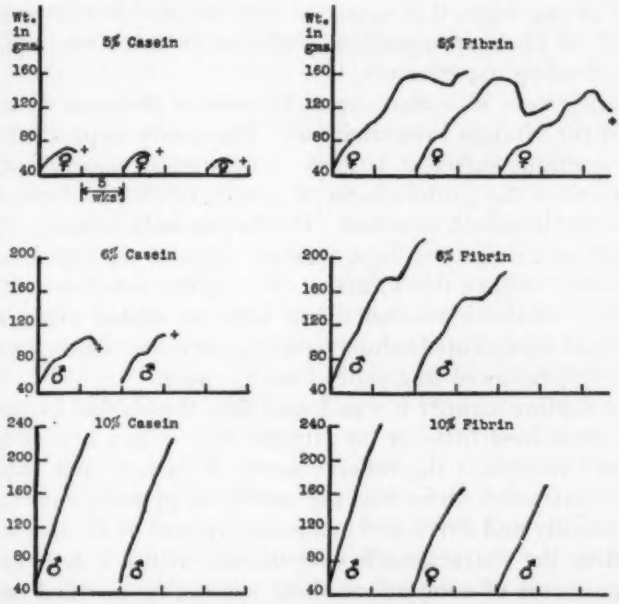

Fig. 1 The comparative growth of rats produced by various levels of alcohol-extracted casein and alcohol-extracted fibrin fed in addition to a basal diet of dried yeast 6, salt no. 40 (Steenbock and Nelson, '23) 4, agar 2, dextrin to make 100 and 0.25 cc. of cod liver oil three times weekly. + indicates death of animal.

Having determined that fibrin could support good growth at comparatively low levels the next step was to ascertain if it produced any toxic symptoms when given in large amounts. For this purpose a diet similar to that used by Parsons ('31) in her studies on the toxicity of egg white was employed. It was composed of the following ingredients expressed in per cent, fibrin (alcohol-extracted) 66, yeast 20, wheat embryo 10,

salt no. 40 (Steenbock and Nelson, '23) 4 and cod liver oil. Four rats (two males and two females) were fed this diet for 12 weeks. At the end of this time the animals appeared perfectly normal. They had increased their weight from an average of 55 gm. to 237 gm. during the experimental period. If the results of Parsons are due to the presence of a toxic factor in egg white it is apparent that no similar substance is present in fibrin in quantities sufficient to interfere with its use in feeding experiments.

Studies were also made upon the use of fibrin in diets intended for vitamin investigations. The crude unpurified material contains sufficient vitamin A to produce nearly normal growth when the protein is fed at a level of 18% and no other source of vitamin A is added. Extraction with alcohol, either by cold percolation or in a Soxhlet apparatus, appeared to completely remove this vitamin. Rats given a ration containing 18% alcohol-extracted fibrin with no added vitamin A developed typical ophthalmia within a period of about 5 weeks which was followed in a short time by death.

In a similar manner it was found that the alcohol-extracted fibrin contained little or no vitamin B_1. When 1% of liver extract [2] furnished the other vitamin B factors and 18% of alcohol-extracted fibrin was the source of protein, rats failed very rapidly and developed symptoms typical of B_1 deficiency, including the characteristic convulsions, within 5 or 6 weeks. For purposes of comparison diets containing purified casein instead of fibrin were fed simultaneously with the fibrin-containing diets used in the vitamin A and B_1 deficiency studies. No differences in the results obtained with the two proteins were detectable.

As fibrin contains considerably less phosphorus than casein it was of interest to study the possibility of using fibrin in rachitogenic diets. Various modifications of the widely used Steenbock and Black ('25) rachitogenic diet no. 2965 were employed in these experiments. In one series of experiments conducted simultaneously three litters of rats at about 25 days

[2] Eli Lilly and Co., no. 343.

of age were divided into four groups and given diets as follows:

Group I. Steenbock-Black diet no. 2965.

Group II. Yellow corn 86, crude fibrin 10, $CaCO_3$ 3, NaCl 1.

Group III. Same as group II except alcohol-extracted fibrin replaced the crude fibrin.

Group IV. Commercial yellow cornmeal 76, wheat embryo 10, alcohol-extracted fibrin 10, $CaCO_3$ 3 and NaCl 1. This last diet is based on a rachitogenic diet previously used in this laboratory (Jones, '34). Within a short time all of the animals with the exception of those of group II began to show definite enlargement of the wrists and at the end of 3 weeks, when all of the animals were bled to death, the enlargements were very pronounced. At the termination of the experiment the blood of each group was pooled and the sera analyzed for calcium by the method of Clark and Collip ('25) and for phosphorus on the calcium-free filtrate by the method of Gunther and Greenberg ('29). The right femur of each animal was removed and the amount of ash in the lipid-free bone was determined and a wrist bone from each animal was examined by the 'line-test' technic. This experiment was repeated as above with two more litters of rats but with a different sample of fibrin. The chemical analyses from both experiments, which are summarized in table 1, and the results from the examination of the wrist bones confirmed the clinical findings. That is, all of the animals except those receiving the unextracted fibrin (group II) showed a marked degree of rickets.

From these experiments it is clear that fibrin can be used as a protein in rachitogenic diets but it first must be extracted with alcohol or similar solvent. Judging from the comparatively small reduction in the amount of phosphorus caused by the extraction it is assumed that the anti-rachitic properties of crude fibrin are due to the presence of vitamin D and not to its higher content of phosphorus. Furthermore, we have frequently observed that if liberal amounts of vitamin D are given in conjunction with a high-calcium rachitogenic diet, the calcium of the blood serum may be considerably above normal.

From table 1 it can be seen that in both experiments the calcium of the serum of the animals receiving the crude fibrin was above 15 mg. per 100 cc.

The fibrin-containing diets used in these experiments contained slightly less phosphorus than the Steenbock-Black diet as the phosphorus content of fibrin is less than that of wheat gluten, and the former protein comprised only 10% of the ration instead of the 20% level at which the wheat gluten is fed. Due to the lower content of phosphorus the fibrin-containing diets should have somewhat greater rachitogenic properties than the Steenbock-Black diet. There is some evidence

TABLE 1

The comparative influence of the various fibrin-containing diets and the Steenbock-Black diet on bone ash and the calcium and phosphorus concentrations of the serum

GROUP	NUMBER OF ANIMALS	GAIN IN WEIGHT [1]	BONE ASH		SERUM	
			Weight [1]	Per cent [1]	Ca	P
		gm.	*mg.*		*mg. per 100 cc.*	*mg. per 100 cc.*
I-A	4	17	17.8	25.5	12.1	2.8
I-B	3	22	24.3	28.4	12.2	2.1
II-A	4	28	40.9	40.6	15.2	6.3
II-B	3	31	48.4	42.9	15.8	5.7
III-A	4	20	16.0	20.8	12.4	1.6
III-B	3	20	21.1	23.8	12.5	2.4
IV-A	5	13	15.7	23.1	12.1	1.9
IV-B	3	19	20.5	24.0	11.5	2.4

[1] Average of all animals of each group.

to indicate that this is true. A comparison of the amount and percentage of ash of the femora of the animals of groups I and III (table 1) show that within each experiment there was more calcification on the Steenbock-Black diet than on the diet containing fibrin. When the fibrin makes up 10% of the ration the growth is comparable to that on the Steenbock-Black diet as is shown by table 1. Additional results on the use of fibrin in rachitogenic diets will be reported in the future.

All the experiments reported above, except the one in which the fibrin was fed at a high level to study its possible toxicity,

were repeated using two different shipments of fibrin. There were no significant differences in the results obtained with the two different samples. Except for the few experiments on B_1 these investigations have not included a study of the possible use of fibrin in work concerning the vitamin B complex. It is highly probable, however, that it may prove useful as casein contains rather large amounts of these factors which are not easily removed.

SUMMARY

Crude beef-blood fibrin as the only source of protein in a synthetic diet supports growth at a level comparable to that of casein. Fibrin can be extracted with organic solvents as readily as casein. With water it swells more than casein. It can be easily dried, ground and incorporated into synthetic diets. Before purification beef-fibrin contains liberal quantities of vitamins A and D which can be removed by extraction with alcohol. Alcohol-extracted fibrin is practically free of vitamin B_1. Fibrin is low in phosphorus and consequently can be used in rachitogenic diets. As fibrin is available at a price approximately the same as casein it may be used in synthetic diets when casein is not suitable.

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LACK OF VITAMIN C IN THE DIET AND ITS EFFECT ON THE JAW BONES OF GUINEA PIGS[1]

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TWO FIGURES

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INTRODUCTION

While working with the teeth of guinea pigs which had been kept on the Sherman, LaMer and Campbell ('22) vitamin C free diet and others kept on the same diet with different vitamin C supplements it was noticed that some of the jaws had dark circular areas on the exterior surface of the mandible at the bases of the cheek teeth, and others were without these areas.

PURPOSE

The purpose of this experiment was to discover the nature of these areas and find if possible a correlation between them and the diet used.

REVIEW OF LITERATURE

Dental caries, alveolar resorption and irregularities of the teeth themselves were produced in guinea pigs fed on a diet without vitamin C (Howe, '20, '21; Höjer, '26; Key and Elphick, '31; and others).

Decalcification of the teeth and some parts of the bones has been reported in guinea pigs as a result of scorbutic feeding (Howe, '22; Zilva and Wells, '19; Höjer and Westin, '25; Key and Elphick, '31; and Dann and Cowgill, '35).

[1] Contribution no. 161, department of zoology and no. 75, department of home economics.

On the other hand it has been claimed that "no apparent morphological change takes place in the hard tissues already formed" (Fish and Harris, '34).

The formation of the teeth in the guinea pig and the formation of bone in which the teeth are located take place at about the same time (Harman and Smith, '36).

MATERIALS AND METHODS

All animals used in this experiment were classified into three groups which were fed as follows: 1) Vitamin C free diet (Kramer, Harman and Brill, '33)—negative controls. 2) Vitamin C free diet plus 3 ml. fresh orange juice daily per 300 gm. body weight. 3) (a) Vitamin C free diet plus greens ad libitum. (b) Ibsen diet (Harman and Prickett, '32) —positive controls.

The Ibsen diet is composed of water, alfalfa hay, greens and a rolled oats mixture. The greens consist of fresh alfalfa in summer and sprouted oats in winter. The rolled oats mixture is as follows:

	Pounds
Rolled oats	50
Wheat shorts	1½
Skimmed milk powder	1½
Tankage	½
Bone meal	½
Table salt	½
100 D brewer's yeast	½

Some animals died and others were killed at stated times. The jaws were preserved in 95% alcohol.

Observations

The dark areas at the bases of the cheek teeth were irregularly circular in shape and varied from 2 mm. to 2½ mm. in diameter (fig. 1). They were so soft that they could be pierced easily with a needle. The bone had the appearance of a sponge. In extreme instances the entire area appeared as a hole.

Usually the jaws having the dark areas were crumbly in the external alveolar part of the mandible in the region of the cheek teeth. More often this condition was present near the posterior cheek teeth but in some instances the entire external alveolar area was so brittle and there was so little hard substance that there was scarcely enough bone remaining to form sockets for the teeth (fig. 2).

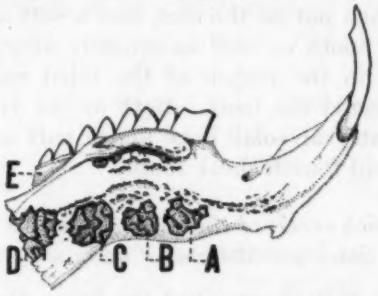

Fig. 1 Drawing of left lower jaw of T-18, from caudad lateral surface showing the soft areas at the base of the cheek teeth. A, B, C and D the soft areas at the base of the first, second, third and fourth cheek teeth, respectively. E, alveolar bone.

Fig. 2 Drawing of left lower jaw of T-18 from cephalad lateral surface, showing the condition of the aveolar bone. F, crumbly alveolar area. G, line of the gum.

Negative controls

Fifteen guinea pigs were used as negative controls. The ages of these animals when put on the diet ranged from 20 days to 3 years and 3 months. The length of time on the diet varied between 26 and 52 days. Five of the animals died between 26 and 40 days on the diet. One died at 41 days and another died at 42 days. One was in a dying condition at 41 days when it was killed. Another had been too weak

to stand for 10 days when it was killed after being on the diet for 52 days. Six of the animals were killed at 28 days.

All the animals had either the soft area at the base of one or more cheek teeth or crumbly alveolar bone or both, whether old or young when they were put on the diet, even if they had been on the diet only 28 days.

All the younger animals, those which were 4 months of age or less when put on the diet, had a soft area at the base of each cheek tooth as well as crumbly alveolar bone. The alveolar area in the region of the third and fourth cheek teeth was affected the most. Both of the very old animals had soft crumbly alveolar bone and a soft area at the base of the third and fourth cheek tooth.

Animals which received the basic diet and 3 ml. of orange juice per 300 gm. of body weight

Twenty-two animals received the basic diet plus 3 ml. of orange juice per 300 gm. of body weight. Their ages when put on the diet varied from 21 days to 10 months. None of the animals of this group died while on the experiment. Two of them were pregnant when killed and two gave birth to young a short time before they were killed. With two exceptions the alveolar and basilar portions of the jaws were firm. One animal had a small soft area at the base of the third cheek tooth and another had a small soft area at the base of the fourth cheek tooth. All other parts of the jaws of these animals were firm and strong. Both of these animals were old, 2½ and 3 years, respectively.

Positive controls (a) vitamin C free diet plus greens ad libitum

Of the five animals receiving the vitamin C free diet plus greens ad libitum all were put on the experiment at birth. The length of time on the diet ranged from 14 to 75 days. None of the animals died. One was pregnant when killed. One had a slightly crumbly alveolar bone. All the others had all parts of the mandible firm and strong.

Positive controls (b) Ibsen diet

Of the animals on the Ibsen diet the jaws and teeth of twenty-nine postnatal animals and twenty-seven embryos, whose mothers had been on the Ibsen diet throughout their lives, were examined:

Postnatal animals. Four of the postnatal guinea pigs were selected as parallels of the four animals of the negative controls and the five receiving orange juice which were more than 2 years old. These thirteen animals had received the same kind of food and had had similar treatment. Twenty of the animals on the Ibsen diet ranged in age from 1 day to 3 years. The ages of five of the animals were unknown, but they were adults and were considered old. The time on the diet varied from 14 to 75 days.

There were no soft spots at the base of any of the cheek teeth of the positive controls. In one animal a part of the alveolar bone was not so firm as the others but it was in no sense crumbly like the negative controls.

Embryos. The number of days after copulation is considered as the age of the embryo. The teeth and jaws of twenty-seven embryos whose mothers had been on the Ibsen diet during their entire life, were examined. These embryos were of twenty-one different litters. The age of the embryos ranged from 44 days to 67 days. Younger embryos were not examined since it is at 44 days the jaw bone and the dentine have become ossified (Harman and Smith, '36). With two exceptions both the areas at the bases of the cheek teeth and the alveolar bones were firm. In two embryos, 55 days and 56 days, respectively, the areas at the base of the third and fourth cheek teeth were not so firm as in the other embryos but they were not crumbly like the negative controls.

DISCUSSION

In the foregoing experiment attention was centered upon the variation of the amount of vitamin C in the diet. An attempt was made to have the other factors as nearly alike as possible in the parallel groups. Animals of about the

same ages which had been raised under similar circumstances were started on the different diets at the same time. When available, animals from the same litter were used. Animals of different ages from the newly born to those which were aged formed a part of each group so that the effect of age as a factor could be considered.

The results of this investigation agree with Howe ('20, '21, and '23), Höjer ('26) and Key and Elphick ('31) in that there is alveolar resorption. However, we have found that resorption of the bone of the jaw extends farther than merely the alveoli. In many instances the outer edges of the mandible is a crumbly mass which may be crushed under very little pressure. Contrary to the report of Fish and Harris ('34) we have found that when no vitamin C was added to the diet morphological changes have taken place in the bones of the jaws in the alveolar areas of the mandible and at the base of one or more of the cheek teeth even in animals which were old and which had been on the basal diet for only 28 days. There was not a single exception to this phenomenon. This condition could scarcely be due to the age of the animal since the ages varied from 20 days to 3 years and 3 months. Neither could it be due to the length of the time on the diet since there were the soft areas at the bases of the cheek teeth in one which had been on the diet only 26 days.

The jaw at the base of the two posterior cheek teeth is the first affected. All of the animals on the vitamin C free diet for more than 28 days had a crumbly alveolar area while those which had been deprived of vitamin C in the diet for only 28 days or less were not affected so extensively.

When this same diet was supplemented by 3 ml. of fresh orange juice per 300 gm. body weight there was scarcely a trace of resorption, although in four instances the animals were pregnant during the experiment. No indication of resorption was found in those animals which received a supplement of greens ad libitum. Animals fed the Ibsen diet had firm, strong jaw bones.

CONCLUSION

The data from this investigation show that:

1. In guinea pigs on the Sherman, LaMer, Campbell vitamin C free diet without supplement, there was resorption of bone at the bases of the cheek teeth and along the edge of the alveolar area. This resorption occurred without respect to the age of the animal.

2. Four weeks on the diet was sufficient time to produce a considerable amount of resorption.

3. The addition of 3 ml. of orange juice per 300 gm. body weight gave almost complete protection against resorption.

4. Guinea pigs fed the vitamin C free diet supplemented with greens ad libitum, those on the adequate Ibsen diet and embryos from mothers on the Ibsen diet had firm and strong jaw bones.

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THE UTILIZATION OF ENERGY PRODUCING NUTRIMENT AND PROTEIN AS AFFECTED BY THE PLANE OF PROTEIN INTAKE [1]

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FIVE FIGURES

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The investigation to be discussed is a continuation of two earlier experiments on the same subject, the results of which were presented by Forbes, Swift, Black and Kahlenberg, in brief in 1935, and in full in this journal also in 1935.

In these earlier experiments equicaloric diets containing 10, 15, 20 and 25% of protein were compared in growth, metabolism and body analysis experiments in which twenty-four rats were used as subjects at each of the four planes of protein intake, with quadruplet, litter mate food control. The effects of the progressively greater protein contents of the diets were as follows:

Increase in gain in body weight, at decreased cost in terms of dry matter of food; increase in efficiency of digestion and retention of protein and of energy-producing nutriment; increase in urinary nitrogen at an increasing rate; increase in protein of the body at a decreasing rate; increase in outgo of energy in the urine coincident with decrease in fecal energy, the metabolizable energy remaining practically constant; diminished efficiency in the utilization of food nitrogen; no regular change in amount of fat gained, but usually a decrease in fat gained in proportion to protein gained.

[1] Authorized for publication on August 31, 1937, as paper no. 785 in the journal series of the Pennsylvania Agricultural Experiment Station.

The progressively greater protein contents of the equicaloric diets, having the effect to improve their nutritive balances, were accompanied by no change in the basal heat production per unit of computed surface area, but by diminished total heat production of the animals, as they lived under normal conditions of freedom of activity.

An investigation, in part of similar significance to the one by Forbes and associates, was conducted by Hamilton ('35, '37); and another study in the same general field was reported, in a preliminary way, by Hogan, Johnson and Ashworth ('35), and later, in full, by Johnson, Hogan and Ashworth ('36).

Hamilton commented briefly on his study ('35) while it was still in progress, and later ('37) published an abstract of the complete account of the investigation, the full report having as yet not come to publication.

Hamilton's first reference to results obtained ('35) was that "sufficient data have been obtained to warrant the statement that as the percentage of protein in the diet increases from 4 to 16 per cent, the thermogenic effect decreases," and this same statement was repeated, in effect, in the later abstract of the complete dissertation. It is a fact, however, that the data presented in the abstract referred to do not warrant the above quotation, without qualification, since, as the protein content of the diets increased from 4 to 8%, the thermogenic effect also increased, from 372 calories to 402 calories per gram of the diet.

The term 'thermogenic effects' was used to signify heat production in excess of the basal energy metabolism, and this quota was measured accordingly.

Some of the more important of Hamilton's conclusions are the following:

Increasing the percentage of protein from 4 to 16 per cent in the diet of growing animals increases the growth-promoting value of the diet. Diets containing between 16 and 30 per cent protein are of equal growth-promoting value, and as the protein increases above 30 per cent, the growth-promoting value decreases.

The voluntary 'running' activity of rats is unaffected by the protein content of the diet, until an excess (54 per cent whole egg protein) percentage is present, when the activity is decreased.

The net energy value of diets for growth plus maintenance increases as the percentage of protein increases up to about 16, remains practically constant throughout the range of protein percentages over which the diets are equally well balanced, and then decreases rapidly in diets containing percentages of protein in excess of that of a well-balanced diet (perhaps more than 30 per cent). The explanation of the higher net energy value of well-balanced diets, as compared with diets unbalanced either because of an insufficiency or because of an excess of protein, lies in the smaller thermogenic effects of the well-balanced diets.

The percentages of metabolizable energy used for basal energy expenditures or for total voluntary activity are unaffected by the percentage of protein in the diet.

A basis for the close interpretation of these findings, however, does not exist, since the method of compounding the diets, involving the direct quantitative substitution of protein for starch, changed not only the protein but also the gross and the metabolizable energy of the diets; and since the determination of 'thermogenic effects,' as increases of heat production above the basal energy metabolism, yielded extremely divergent values (135 to 625 calories), because of the different sparing effects of the diets on the katabolism of body substance of the rats in their initial basal status.

In other words these 'thermogenic effects' were of mixed nutritive origin and significance.

In the study of Johnson, Hogan and Ashworth ('36), two planes of protein intake, 10% and about 25%, were compared. The authors concluded, in part, that

> growth was more rapid on the reasonably high protein diets than on like diets inadequate in protein content. These differences were not due to differences in energy lost in the excreta, in the total heat lost, or in the energy gains, but were due to differences in the kind of nutrients stored. The animals on the high protein diets stored more water, protein, and ash than their pair-mates, and thus stored less energy

per unit gain, while those on the low protein diets stored more fat than their pair-mates, and thus stored more energy per unit gain. The net utilization of energy for body gain by all animals was the same.

As with Hamilton's study, the plane of energy intake was not the same for the animals which received the two diets differing in protein content.

On the basis of the results obtained with the animals designated group 4, which afford the best opportunity for comparison, the animals on the high-protein diet received 6.7% more gross energy, and 5.7% more metabolizable energy, and they produced 3.7% more heat and 5.4% more energy gain than did those which received the low protein diet.

PLAN OF EXPERIMENT

The present experiment, like the two earlier ones by Forbes and associates, was a 70-day growth and metabolism investigation, employing the body-balance method, and the open circuit, Haldane, respiration procedure, the subjects being twelve quadruplets of albino rats (six each of males and of females) from the same number of litters, fed with quadruplet, litter mate food control.

In both the earlier and the present studies the problem, the methods of procedure, and the whole experimental set-up were the same, except that, whereas, in the two earlier experiments, the protein contents of the diets were 10, 15, 20 and 25%, respectively, in the present study they were 25, 30, 35 and 45%, respectively. The purpose of the present study was, obviously, to extend the evidence derived from the earlier studies in such manner that the entire series would cover the range of variation of protein contents of the diets from 10% to 45%—that is, from moderate deficiency to great excess of this nutrient.

Four diets were compared at once, with one rat from each quadruplet on each diet, there being as many individuals on a treatment, therefore, as there were quadruplets.

The diets having been compounded to be exactly equicaloric, but differing in protein content, and the initial weights of the animals of each quadruplet being the same, the food was given to the individuals of each quadruplet in equicaloric quantities, determined, for each quadruplet, by the individual animal which ate the least food.

The quantities consumed within quadruplets, therefore, were identical, though the food consumption was unlike for the different quadruplets.

This method of food assignment was designed to be as nearly equitable as practicable, but in the course of its use it becomes somewhat inequitable to those individual animals that have received the more efficient diets, since these favored subjects will have made the larger growth, and will be receiving less food in addition to their maintenance requirements than will those which have received the less efficient diets.

This situation prevents the full expression of the superiority of the better diets, and, therefore, has the effect to render the observed less than the true differences between the diets.

This error, however, is conservative in effect since it minimizes rather than exaggerates the differences in the results obtained.

In view of the method of food assignment employed, which resulted in equal energy intake within but different energy intake among quadruplets, and the observation made that the methods of use of food energy and protein were much affected by the food intake within equal-food-protein groups, the data derived from the quadruplets of rats which ate essentially the same quantity of food in the three experiments were segregated, both in the tables and the graphs, and were given special consideration as the most significant results of the investigation.

Course of the experiment

The investigation progressed, from beginning to end, with remarkable regularity, and without noteworthy incident or departure from plan.

As each quadruplet of rats was given increasing quantities of feed, from day to day, until one rat of the four failed to eat the entire quantity, the results afford a basis for conclusion as to the acceptability, to the rat, of the four diets, of different protein contents.

The numbers of such refusals, by the rats which received the diets of different protein contents, were as follows:

Per cent protein in diets	*Food refusals*	
	First 3 weeks	Last 4 weeks
25	59	81
30	66	111
35	33	48
45	152	65

These data show that the rats accepted the 35% protein diet most readily, and the 45% protein diet least readily, during the first 3 weeks, but that this difference existed only to an extent of doubtful significance during the last 4 weeks of the experiment. The order of the total number of refusals, for the 7 weeks, however, was as during the first 3 weeks. No especial importance is ascribed to these data; but it seems to be a fact that the 35% protein diet was the most acceptable to the animals. This response should be regarded more as an expression of final nutritive effect than of palatability.

Composition of diets

The percentage composition of the four diets was as in table 1.

It will be observed that these diets contained only 2.0% of butterfat, as a constant constituent, instead of 10.0%, as in the experiments discussed in the earlier paper; while the Crisco varied in percentage between 2.746 and 9.128, instead of between 0.0 and 5.102, as in the earlier experiments.

These differences came about as incidents in the computation of the four diets, each to be of the same energy value, but to contain the larger proportions of protein than as in the diets fed in the earlier experiments.

Then, the vitamin A content was safeguarded by the inclusion of carotene, in oil, as explained in the note below table 1. The gross energy value of these four diets was 4696 calories per gram.

TABLE 1

Composition of diets

	NO. 1	NO. 2	NO. 3	NO. 4
	%	%	%	%
Cellu-flour	4.000	4.000	4.000	4.000
O. and M. salt mixture	4.000	4.000	4.000	4.000
Na Cl	1.000	1.000	1.000	1.000
Yeast [1]	6.000	6.000	6.000	6.000
Butterfat	2.000	2.000	2.000	2.000
Dextrin	49.374	45.360	41.345	33.316
Casein	24.498	30.108	35.718	46.938
Crisco	9.128	7.532	5.937	2.746

[1] The yeast was a mixture of 5 parts brewer's yeast and 1 part irradiated yeast.

NOTE: Carotene, in oil, was added to each diet, in the proportion of 2.5 gm. to each 7500 gm. of other constituents. This supplied 1.4 U.S.P. units of vitamin-A-equivalent per gram of food.

TABLE 2

Food eaten, and average quantities, character and gross efficiency of gains in weight

PLANE OF PROTEIN INTAKE	FOOD EATEN (DRY MATTER)	GAIN IN BODY WEIGHT[1]	FOOD (DRY MATTER) PER GRAM BODY GAIN	NITROGEN OF BODY GAIN	FAT GAINED	FAT GAINED PER GRAM N GAINED
% protein	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
25	451	116.70	3.9 ± 0.11	3.79	21.7	5.7 ± 0.25
30	451	112.87	4.0 ± 0.13	3.67	20.5	5.6 ± 0.26
35	451	112.34	4.0 ± 0.11	3.70	19.6	5.3 ± 0.24
45	451	104.76	4.3 ± 0.13	3.45	17.7	5.1 ± 0.25

[1] Contents of alimentary tract removed.

NOTE: Each datum is an average representing twelve animals on a continuous metabolism experiment during 10 weeks.

Body gains

Referring to table 2, and to figure 1, it is clear that, with the same food intake, the progressively greater protein contents of the diets above 25% resulted in diminished gain in body weight. Such greater protein contents of diets as did not serve to improve the efficiency with which the food protein

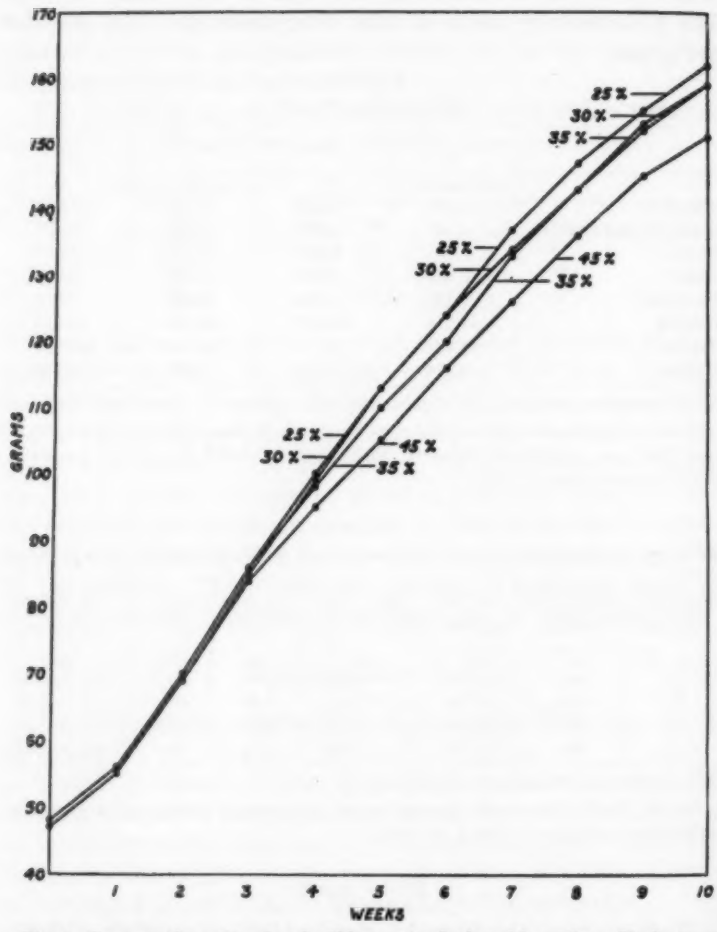

Fig. 1 Representing growth of albino rats during 10 weeks. Each curve represents twelve rats, each such group having received the same average energy intake, but a different percentage of protein in the diet.

was retained were definitely disadvantageous, because of the lower metabolizability of protein than of non-nitrogenous organic nutrients.

The odds that the average gain in body weight from the 25% protein diet was greater than that from the 45% protein diet were more than 10,000 to 1.

TABLE 3

Distribution of average total food energy as affected by the plane of protein intake

PLANE OF PROTEIN INTAKE	FOOD ENERGY	FECES	DIGESTED	URINE	METABOLIZED	BODY GAIN	BODY GAIN AS PROTEIN	BODY GAIN AS FAT	HEAT
Representing all animals fed, experiments 1 and 2									
%	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*
10	2119.8	181.4	1938.4	42.9	1895.5	225.3	80.9	144.4	1670.2
15	2119.8	170.0	1949.8	54.4	1895.4	261.0	122.5	138.5	1634.4
20	2119.8	150.2	1969.6	71.6	1898.0	281.5	134.9	146.6	1616.5
25	2119.8	140.6	1979.2	91.3	1887.9	285.9	139.0	146.9	1602.0
Representing all animals fed, experiment 3									
25	2261.3	161.2	2100.1	99.4	2000.7	331.9	129.1	202.8	1668.8
30	2261.3	156.4	2104.9	120.7	1984.2	319.5	126.8	192.7	1664.8
35	2261.3	149.4	2111.9	142.4	1969.5	311.2	127.3	183.9	1658.3
45	2261.3	140.1	2121.2	187.2	1934.0	284.5	118.5	166.0	1649.5
Representing animals selected for uniform food intake, experiments 1 and 2									
10	2137.1	182.6	1954.5	43.6	1910.9	212.3	77.2	135.1	1698.6
15	2137.1	166.1	1971.0	54.5	1916.5	257.1	118.9	138.2	1659.4
20	2137.1	150.7	1986.4	72.2	1914.2	274.8	129.4	145.4	1639.4
25	2137.1	140.8	1996.3	91.9	1904.4	297.6	138.5	159.1	1606.8
Representing animals selected for uniform food intake, experiment 3									
25	2141.0	151.2	1989.8	95.9	1893.9	304.6	117.6	187.0	1589.3
30	2141.0	151.2	1989.8	115.4	1874.4	278.1	115.5	162.6	1596.3
35	2141.0	143.1	1997.9	136.0	1861.9	282.0	114.2	167.8	1579.9
45	2141.0	131.8	2009.2	178.2	1831.0	255.0	111.2	143.8	1576.0

It is also shown, in table 2, that progressively greater protein contents of the equicaloric diets, from 25 to 45%, caused decreased gains of nitrogen and of fat, but a study of the proportion of fat gained to nitrogen gained, on the basis of the data in this table and those presented in table 3, did not reveal consistent effects of the plane of protein intake on the composition of the body gains.

The average ratios of protein gained to water gained, for the groups which received the diets differing in protein content, were identical. The data are not presented.

Distribution of energy intake

In the remainder of the present paper the data from the experiments of the earlier study are combined, for purposes of discussion, with the new data from the third experiment, in order to make a unified presentation of the results of the entire investigation, covering the range of variation between 10% and 45% in the protein of the diets.

In table 3 is represented the distribution of the total quantities of food energy received by the rats as affected by the plane of protein intake, this table being divided into four sections, the first representing all animals fed in experiments nos. 1 and 2 (the published experiments); the second, all animals fed in experiment no. 3 (the new experiment); the third, a selection of quadruplets of animals which received the same quantity of food, in experiments nos. 1 and 2; and the fourth, a similar group of quadruplets from experiment no. 3, selected for essentially the same uniform food intake as that representing experiments nos. 1 and 2, in the third section of the table.

These data for selected animals, given in the third and fourth sections of the table, represent five quadruplets of rats from the first, and seven from the second experiment, the average energy intake during the 10 weeks in both groups being 2137 Calories, and six quadruplets from the third experiment, the average energy intake of which was 2141 Calories.

The data comprising the first two sections of table 3 are graphically presented in figure 2, and those in the last two sections are represented in figure 3.

The breaks in the continuity of the curves in figure 2 result mainly from differences in the average plane of nutrition, but apparently also to a slight extent from the differences in the composition of the diets, which have been explained.

These breaks, therefore, are largely eliminated by the selection of quadruplets of animals for uniform food intake, as represented in figure 3; but this selection did not serve perfectly to smooth out the curves representing some of the data,

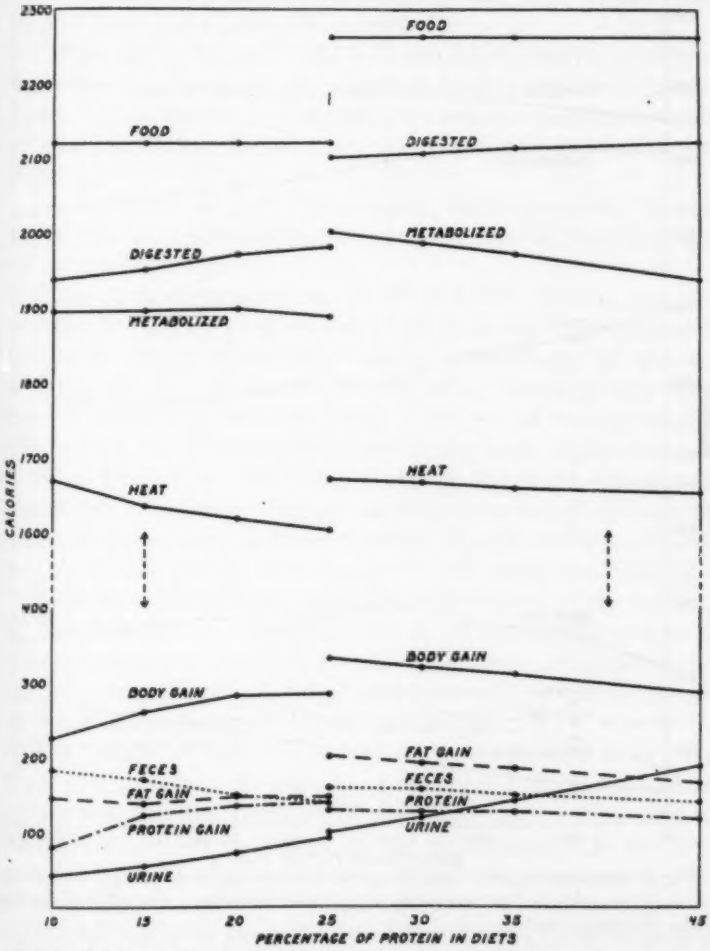

Fig. 2 Representing the distribution of the average food energy of all animals fed, as affected by the plane of protein intake.

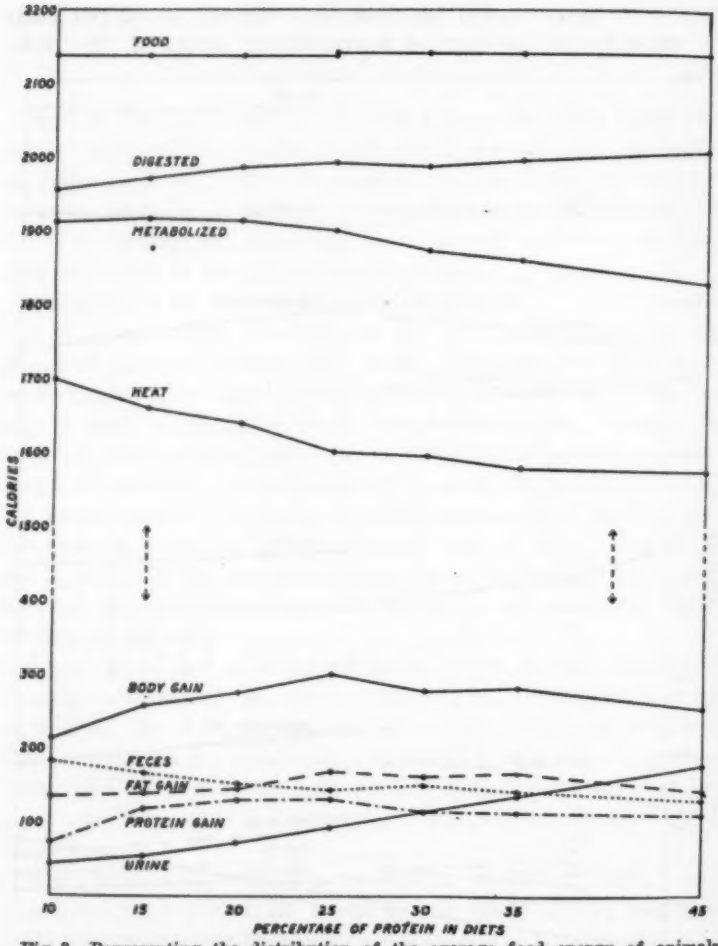

Fig. 3 Representing the distribution of the average food energy of animals selected for uniform food consumption, as this distribution was affected by the plane of protein intake.

especially those relating to the fat gain and the protein gain, since the composition of the body increase of rats is much affected by the individuality of the animal, and since the selected data represent comparatively few individuals—only half of the total number of animals fed.

Referring to figure 3, which is considered more significant than figure 2, the curves representing fecal energy and digested energy show that with progressively greater protein contents of the diets there were increasing quantities of food energy digested.

Digestibility, as here represented, was 'apparent' digestibility, that is, without separate consideration of fecal nitrogen of metabolic origin.

With diets containing 10, 15, 20 and 25% protein the progressively smaller proportions of fecal energy approximately balanced the progressively larger proportions of urinary energy, the metabolizable energy being virtually the same for all diets, and the increasing quantities of energy utilized for body gain balancing the diminishing heat production.

With more than 25% of protein in the diets the greater quantities of urinary energy more than counterbalanced the smaller quantities of fecal energy—the quantities of both metabolizable energy and energy of body gain diminishing.

With these diets containing 25 to 45% of protein the rapidity of the decrease in the quantities of the resulting products, corresponding to the progressively greater proportions of protein, was first in metabolizable energy, second in energy of body gain, and third in heat production.

The values for metabolizable energy were computed as gross food energy minus the energy of the feces and urine, without correction for the fraction of the body gain which is non-metabolizable—in the sense of not being available for heat production. In some relations, and for some purposes, such a correction is made in studies conducted at this institute.

As indicative of the validity of the observations of gain of energy, the odds that the gain from 25% protein diet was greater than that from the 45% protein diet were 5000 to 1.

The outgo of energy in the form of heat (computed as the gross energy of the food minus the energy of the excreta and of the body gain) diminished, with a diminishing rate of decrease, as the protein contents of the diets increased from 10 to 45%.

The heat which represented voluntary activity was not separated from that which represented energy expense of food utilization, but the basal heat production seems to have been unaffected by the plane of protein intake. The curve of heat production can best be interpreted, therefore, in general terms, simply as one of the effects of the differences in nutritive balance, the decrease in heat from diets containing 10, 15, 20 and 25% protein representing mainly improved nutritive balance, and the slight continuing decrease of heat from diets containing 30, 35 and 45% protein representing mainly diminished metabolizability (due, in this case, to increased urinary energy) of the diets.

The foregoing observation on the basal heat production in relation to the protein contents of the diets is based on determinations of this value as reported in the earlier paper, by Forbes and associates, and on further determinations made in connection with the present (third) experiment, these last-mentioned determinations having been made after the rats had been on experiment for 6 weeks.

The average values found in these last determinations, expressed as calories per computed square meters of body surface, per hour, were as follows: for the 25% protein rats, 32.9 Calories; for the 30% protein rats, 33.4 Calories; for the 35% protein rats, 34.0 Calories; and for the 45% protein rats, 33.7 Calories.

The calculated odds that the differences between these averages are statistically significant are as follows: 25 and 30% protein, 3:1; 25 and 35% protein, 293:1; 25 and 45% protein, 22:1; and 30 and 35% protein, 4:1. The difference between the values for the rats which received the diets containing 25 and 35% protein being only 3.25% of the smaller value, the significance of this difference seems highly questionable, in spite of the computed odds, especially because

of the indefiniteness of the so-called basal metabolism as a physiological quota.

The distribution of the food energy on a percentage basis is presented in table 4, these data being in harmony with the general trends observed in the corresponding data on the absolute basis.

The remarkable fact made clear by the evidence presented is that the heat production is as little affected as it was found to be by the plane of protein intake.

TABLE 4

Percentage distribution of average total food energy by animals selected for uniform intake of food energy

PLANE OF PROTEIN INTAKE	FECES	DIGESTED	URINE	METABOLIZED	BODY GAIN	BODY GAIN AS PROTEIN	BODY GAIN AS FAT	HEAT
				Experiments 1 and 2				
%	%	%	%	%	%	%	%	%
10	8.5	91.5	2.0	89.5	9.9	3.6	6.3	79.6
15	7.8	92.2	2.6	89.6	12.0	5.6	6.4	77.6
20	7.1	92.9	3.4	89.5	12.9	6.1	6.8	76.6
25	6.6	93.4	4.3	89.1	13.9	6.5	7.4	75.2
				Experiment 3				
25	7.1	92.9	4.5	88.4	14.2	5.5	8.7	74.2
30	7.1	92.9	5.4	87.5	13.0	5.4	7.6	74.5
35	6.7	93.3	6.4	86.9	13.2	5.3	7.9	73.7
45	6.2	93.8	8.3	85.5	11.9	5.2	6.7	73.6

The significance of the values for heat production, so far as they affect net energy, is that net energy is not a characteristic function of individual nutrients, or of individual foodstuffs (except as these may be fed alone), but is a function of the entire diet—a point of view which was expressed by Forbes, in 1929, and which has since been discussed by Forbes, or by Forbes and associates, in numerous publications, but especially in papers cited as by Forbes, Braman, Kriss and Swift ('31), Forbes ('33 a, b) and Forbes, Braman, Kriss and Swift ('33).

Distribution of nitrogen intake

In table 5 are set forth the data for distribution of food nitrogen as affected by the plane of protein intake, these observations, for all animals fed, being graphically illustrated

TABLE 5

Distribution of average total food nitrogen as affected by the plane of protein intake

PLANE OF PROTEIN INTAKE	FOOD NITROGEN	FECES	DIGESTED	URINE	BODY GAIN
Representing all animals fed, experiments 1 and 2					
%	gm.	gm.	gm.	gm.	gm.
10	7.23	0.86	6.37	3.82	2.21
15	10.79	0.90	9.89	6.12	3.35
20	14.28	0.96	13.32	9.12	3.71
25	17.88	1.01	16.87	12.39	3.80
Representing all animals fed, experiment 3					
25	19.12	1.21	17.91	14.34	3.79
30	23.55	1.29	22.26	17.97	3.67
35	27.21	1.31	25.90	21.85	3.70
45	34.62	1.44	33.18	29.20	3.45
Representing animals selected for uniform food intake, experiments 1 and 2					
10	7.28	0.86	6.42	3.87	2.14
15	10.87	0.92	9.95	6.18	3.29
20	14.41	0.96	13.45	9.29	3.58
25	18.04	1.02	17.02	12.51	3.77
Representing animals selected for uniform food intake, experiment 3					
25	18.10	1.11	16.99	13.90	3.43
30	22.30	1.28	21.02	17.13	3.35
35	25.76	1.29	24.47	20.93	3.33
45	32.78	1.33	31.45	27.68	3.25
Exp's 1, 2, 3 Av. 25 (selected)	18.06	1.05	17.01	12.97	3.66

in figure 4; and for animals selected for uniform food consumption in figure 5.

An interesting arithmetical principle is exemplified, in this figure, by the nearly parallel lines representing food nitrogen and digested nitrogen, and by the nearly horizontal line representing fecal nitrogen.

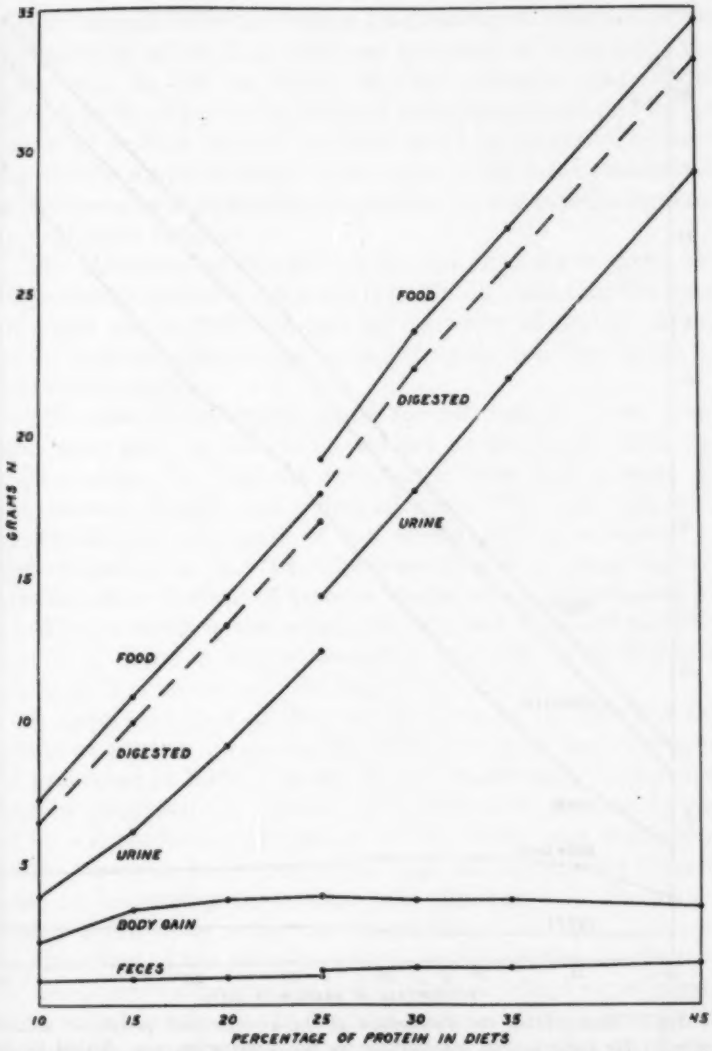

Fig. 4 Representing the distribution of the average food protein of all animals fed, as affected by the plane of protein intake.

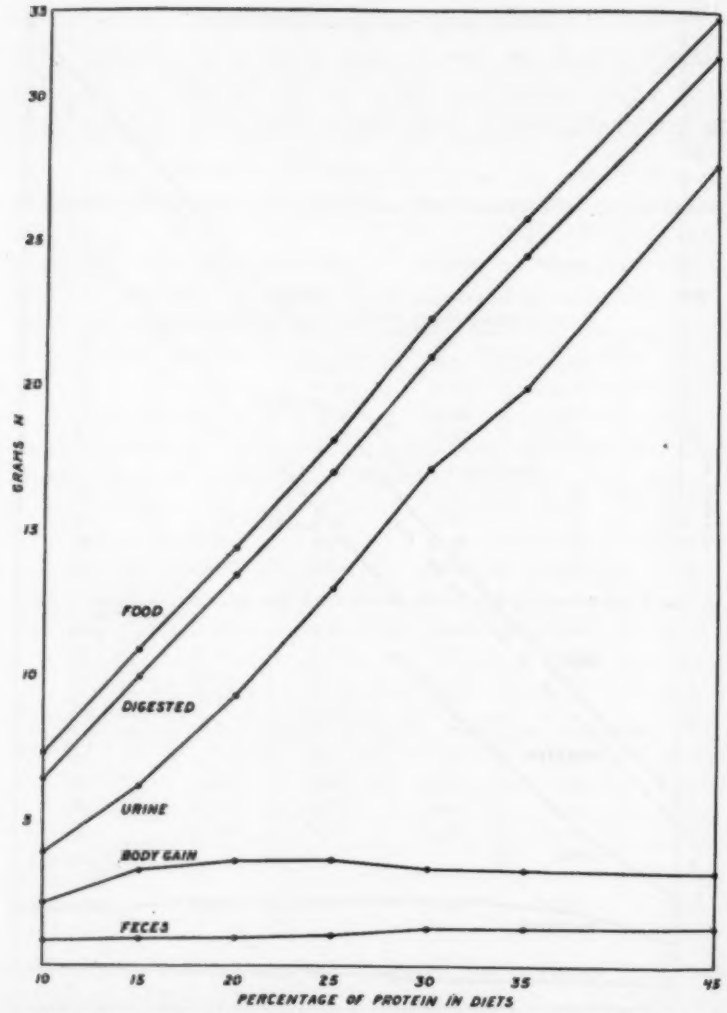

Fig. 5 Representing the distribution of the average food protein of animals selected for uniform food consumption, as this distribution was affected by the plane of protein intake.

The natural inference from a glance at these lines is that the digestibility of the food nitrogen increased at essentially the same rate as did the intake of food nitrogen, and, therefore, that the digestibility was not significantly affected by the plane of protein intake; but this would be in error, because the nearly constant outgo of nitrogen in the feces constituted a significantly diminishing proportion of the rapidly increasing nitrogen intake.

The substance of the matter is that the values given for digestibility were for apparent digestibility, and that the fecal nitrogen was so little affected by the plane of protein intake as to indicate that it (the fecal nitrogen) was nearly all of metabolic origin.

The sums of the values given for nitrogen of feces, urine and body gain, in this table, do not agree exactly with the values given for food nitrogen, since these values were all determined directly, and independently. These discrepancies represent the resultants of the slight, inevitable errors of determination of the four above-mentioned nitrogen values. Among these factors of error a visible one is contributed by shed hair, which is not accounted for, and a second possible factor of error is loss of ammonia from the urine between the time it is voided and the time it is collected.

A significant view of this consideration of the effect of the plane of protein intake on the distribution of food nitrogen is presented in table 6, in which it is shown that corresponding to progressively greater proportions of protein in the diets a diminishing percentage of the intake was eliminated in the feces; an increasing percentage was apparently digestible; an increasing percentage was eliminated in the urine; and a diminishing percentage was utilized for body increase.

The effect of the plane of protein intake on the distribution of the urinary constituents in the whole series of experiments is revealed in table 7. In the first and second experiments, in which the percentages of protein in the diets were 10, 15, 20 and 25, respectively, the Calories per gram of nitrogen in the urine diminished from 10.6 to 7.4; and in the third experiment, with percentages of protein in the diets of 25, 30, 35

and 45, respectively, and a plane of food intake slightly higher than in the first two experiments, the Calories per gram of urinary nitrogen diminished from 6.9 to 6.4.

The reason that the values, in table 7, for the grams of urinary nitrogen resulting from the 10% and the 15% protein rations, differ slightly from the corresponding values in table

TABLE 6

Percentage distribution of average total food nitrogen by animals selected for uniform intake of food energy

PLANE OF PROTEIN INTAKE	FECES	DIGESTED	URINE	BODY GAIN
		Experiments 1 and 2		
%	%	%	%	%
10	11.8	88.2	53.2	29.4
15	8.5	91.5	56.9	30.3
20	6.7	93.3	64.5	24.8
25	5.7	94.3	69.3	20.9
		Experiment 3		
25	6.1	93.9	76.8	19.0
30	5.7	94.3	76.8	15.0
35	5.0	95.0	81.3	12.9
45	4.1	95.9	84.4	9.9

TABLE 7

Relationship of nitrogen and energy in urine with different levels of protein in the diet

PLANE OF PROTEIN INTAKE	NITROGEN OF URINE	ENERGY OF URINE	CALORIES PER GRAM OF NITROGEN IN URINE	PLANE OF PROTEIN INTAKE	NITROGEN OF URINE	ENERGY OF URINE	CALORIES PER GRAM OF NITROGEN IN URINE
%	*gm.*	*Cal.*		%	*gm.*	*Cal.*	
10	3.88	41.0	10.6	25	14.34	99.4	6.9
15	6.18	54.9	8.9	30	17.97	120.7	6.7
20	9.11	71.5	7.8	35	21.85	142.4	6.5
25	12.39	91.3	7.4	45	29.20	187.2	6.4

5 is that during the use of these data, from the earlier paper on the same subject as the present one, errors—apparently of transposition—were discovered in two individual data contributing to the above-mentioned averages. These two erroneous values were excluded from the averages given in table 7.

The significance of these data is that with progressively greater protein contents of the diets there is a decrease in the proportion of nitrogenous compounds of endogenous origin, and an increase in the proportion of urea, in the urine. The Calories per gram of nitrogen in urea being 5.47, and the decrease in Calories per gram of nitrogen, as the percentage of protein in the diet increased from 35 to 45 being only from 6.5 to 6.4, it is obvious that further possible increase in the protein of the diet could produce but little further decrease in the Calories per gram of nitrogen in the urine.

SUMMARY

Corresponding to progressively greater protein contents of equicaloric diets, from 10 to 45%, were increased digestibility and decreased metabolizability of food energy; decrease in heat production at a diminishing rate of decrease; increase in energy of urine; increase in gain in body weight and in energy of body gain until the optimum proportion of protein in the diet was reached, and, with further increase in protein, slight decreases in rate of gain in weight, energy, nitrogen and fat, and in fat gained per gram of nitrogen gained.

The nutritive balance of the diets as sources of energy was obviously improved, corresponding to their progressively greater protein contents from 10% to 25%, as evidenced by marked increase in energy of body gain; and approximately equal decrease in heat production—the metabolized energy remaining virtually unchanged.

Corresponding to further increase in the protein contents of the diets from 25 to 45%, the nutritive balances of the diets as sources of energy were slightly impaired, as evidenced by appreciable decrease in the quantity of energy utilized for body gain, due to more rapid decrease in metabolizable energy than in heat production—the decrease in metabolizable energy resulting from an increase in energy of urine which exceeded the slight decrease in energy of feces.

Corresponding to progressively greater protein contents of the diets from 10 to 45%, there was a slight decrease in the proportion of food nitrogen appearing in the feces; a considerable increase in the proportion appearing in the urine; and first a marked increase, followed by a marked decrease, in the proportion utilized for body gain.

The plane of protein intake did not materially affect the basal energy metabolism.

The results tend to sustain the idea that the specific dynamic effects of protein, carbohydrate and fat, as their relative values are ordinarily understood, do not apply in relation to the mixed diets of nutritive practice; and the idea that neither individual nutrients nor individual feeding stuffs express their maximum, normal, nutritive values except as components of complete diets.

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STUDIES ON THE EFFECTS OF A BOVINE BLINDNESS-PRODUCING RATION UPON RABBITS[1]

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THREE FIGURES

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Blindness of cattle caused from stenosis of the optic foramen seems to arise from nutritional sources. This type of blindness has been reported by Huffman ('29), Walker ('36) and others. Huffman and co-workers associated the blindness with poor quality roughage and found that cod liver oil was effective in its prevention, although Moore, Huffman and Duncan ('35) found that 10 to 20 mg. of carotene daily failed to protect calves. A somewhat similar type of blindness has been found to accompany the feeding of cottonseed meal and poor roughage. Reed, Huffman and Addington ('28) obtained it on rations when cottonseed, or linseed meal, was fed with wheat straw as the roughage. Halverson and Sherwood ('30) suggested a vitamin A deficiency but were unable to entirely supplement cottonseed meal rations for dairy cows by the use of 27% alfalfa hay. Bechdel and Skaggs ('35) were unable to arrest the blindness by the administration of large doses of carotene. Gallup, Kuhlman and Weaver ('36) suggested that some nutritive factor other than vitamin A or D was responsible since cod liver oil was superior to carotene or tomato juice and vitamin D. de Schweinitz ('32) considers the problem to be a genetic factor. Huffman ('29)

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and Walker ('36) have shown that vitamin D is not involved. The latter author states that "vitamin A cannot be entirely ruled out," although his evidence suggested that some other factor might be responsible for the blindness.

Since considerable uncertainty exists as to the cause of the stenosis of the optic foramen an attempt to produce and study it with laboratory animals was undertaken.

EXPERIMENTAL

A series of experiments was made. In a preliminary experiment no. 361 an attempt was made to produce in the rabbit this blindness which has been referred to as atypical blindness. A second experiment no. 362 was planned to study the efficiency of various methods of supplying carotene as a source of vitamin A, while a third experiment no. 363 was planned to re-check the efficacy of carotene for preventing blindness on the Walker ration and to control the effects of the carotene carrier.

The basal ration used in this series of experiments was one that was used by Walker ('36) in producing optic foramen stenosis in calves. It was composed of white corn, 22 parts; linseed oil meal, 23 parts; wheat middlings, 11.5 parts; oat mill feed, 40 parts; ground limestone, 3.0 parts; iodized salt, 0.5 parts, and irradiated yeast, 0.1 part.

Experiments were made with both guinea pigs and rabbits and identical results were obtained with each species. However, the routine difficulties of supplying ascorbic acid to care for the vitamin C requirements and the eccentricity of the guinea pig led to its elimination for these studies and only the results with rabbits are reported here. Four young rabbits were used in experiment no. 361. All received the basal ration. One received in addition 150 micrograms of (SMA) carotene daily per kilogram of body weight fed in Wesson oil as a carrier and the second received similarly 30 micrograms of carotene daily per kilogram of body weight. Twenty young rabbits were used in experiment no. 362 and divided into five lots as follows:

Lot I. Basal ration only.

Lot II. Basal ration plus 2% Wesson oil containing sufficient carotene to furnish 1.1 microgram of carotene per gram of feed.

Lot III. Basal ration plus a daily subcutaneous injection of carotene suspended in water. Dosage 44 micrograms per kilogram body weight. This dosage was adjusted as indicated later.

Lot IV. Basal ration plus 2% aerated cod liver oil.

Lot V. Basal ration plus 1.1 microgram carotene per gram of feed evaporated from an ether solution.

The aerated cod liver oil used in lot IV was prepared by the usual method used in this laboratory (Rupel et al., '33) for the purpose of destroying vitamin A.

In experiment no. 363 young rabbits were placed on the basal ration plus ample carotene for 1 week and then alotted to four lots of three animals each on the basal ration. Lots I and II received added carotene, I in the form of oral administration of carotene in Wesson oil at the rate of 50 micrograms per kilogram of body weight, and II was given the carotene by adding 2% Wesson oil carrying enough carotene to give 0.6 micrograms per gram of feed. Lots III and IV were on the basal ration only but in the latter 2% of unfortified Wesson oil was added.

The rabbits used in these experiments were weanlings of the American white, chinchilla or Himalayan breeds. The harshness of the ration caused some difficulty with the rabbits during the first weeks and considerable mortality was experienced. Animals of the same sex were used in experiments nos. 361 and 363. In experiment no. 362, one male and three females were placed in each lot.

RESULTS

This series of experiments gave consistently concordant results in the following respects: Stenosis of the optic foramen could not be produced in the rabbit, no doubt due to the anatomical arrangement of the skull. Blindness and ataxia developed in the rabbit on the unsupplemented ration with strikingly similar symptomology to that of the growing calves. Growth was retarded on the basal ration. Supplementary additions of carotene from the beginning of the experiment in doses of 50 or more micrograms per kilogram of body

weight completely prevented the development of the blindness and ataxia. This level permitted normal growth, health and vigor over an 8-month period, and it was adequate for pregnancy and gestation. The mode of administration of the carotene was without influence provided the dose was ample at all times. Wesson oil, aerated cod liver oil, or lactoflavin failed to prevent the disease. Once the syndrome was distinctly apparent doses of 35 or 70 micrograms of carotene (SMA Company crystalline carotene) per kilogram of body weight per day were ineffective in arresting or remedying the progress of the disease. The basal ration alone produced 100% mortality.

The symptoms shown by the rabbits developing this syndrome were strikingly similar in all respects to those produced in calves. They may begin as an involvement of locomotion, equilibrium, or sight. In approximately 50% of the cases ataxia and loss of equilibrium developed concurrently with or before the eye symptoms began. Usually, however, the first symptoms appeared in the eye with a fleeting or persistent ophthalmia, hyperemia, and congestion, followed very soon by an erosive dry keratitis progressing rapidly from Bitot's spots to opacity and blindness. The keratitis observed was the interstitial type which gave a ground glass appearance to the cornea as shown in figure 1. The light reflex was sluggish and somewhat disturbed. This condition was accompanied by progressive ataxia and loss of equilibrium when posture was disturbed quickly. This was observed most frequently when the animals were lifted free of the floor. Convulsive efforts were made in an attempt to regain 'position' although no definitely clonic seizures were seen. Infrequent stiffness and partial paralysis of the legs occurred, most frequently noted in the front quarters. The normal position of the head was often lost. Persistent diarrhea and salivation accompanied the disorder, particularly in the later stages.

Growth was retarded and in severe cases loss of weight occurred as shown in figure 2. The administration of 70

micrograms of carotene to the animals in experiment no. 361, lot III, failed to prevent loss of weight and other symptoms. Two per cent aerated cod liver oil was ineffective in maintaining normal growth and preventing loss of body weight. Likewise 2% Wesson oil was without potency unless fortified with carotene. A flavin concentrate given in large doses for

Fig. 1 A rabbit from experiment 363 lot III which received the basal ration only. Note the ground glass appearance of cornea.

a week had no beneficial effect. The daily injection of 44 micrograms of carotene suspended in water was ineffective for growth promotion and prevention of the development of the syndrome. At point A, figure 2, lot III, experiment no. 362, four massive doses (15,000 units) of vitamin A were given to one animal over a period of 8 days and another animal was given a daily injection of 300 γ of carotene for 1 week. Thereafter both animals were continued on a daily

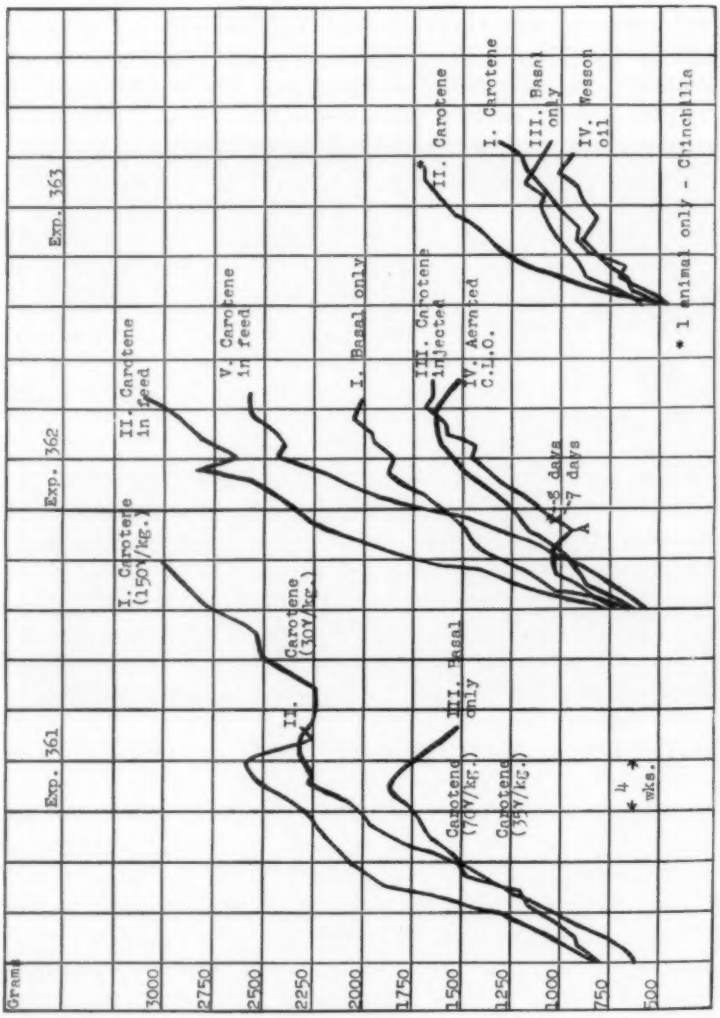

Fig. 2 Growth curves of rabbits on experimental diets.

injection of 100 γ per kilogram of body weight. Growth, which had stopped completely, was resumed in both cases although at a retarded rate. This was accompanied by a recession of symptoms and gradual return to normal.

Pathological analysis of the first experiment was not attempted. The animals in the other experiments were utilized for histological material and portions of the central and peripheral nervous system were taken for microscopic study as well as portions of the liver, spleen, kidney and suprarenals. Examination of the brachial plexus area, the optic and sciatic nerves, were made with the polarizing microscope and with appropriately fixed material by the rapid Marchi method of Geist ('35), Sudan III, silver, and H and E. In general the results obtained with the Marchi method, Sudan III, and the polarizing microscope gave concordant results on the peripheral nerves. The latter was found to be of little use in the study of the optic nerve and cord. A total of eight animals was studied.

Six of these showed kidney damage in the glomeruli and tubules. The changes in the kidney were early mild parenchymatous degeneration in the proximal convoluted tubules, a general tendency for connective tissue response with focal interstitial proliferation (fig. 3), inflammation and congestion with some oedema. Attention is called to the marked similarity of the histopathology to that reported by Reed and associates ('28). Metaplasia of the renal pelvis epithelium was observed in two cases. Five livers showed a reaction in the von Kupfer cells and hepatic congestion. In the former case there seemed to be sufficient hypertrophy to bring the von Kupfer cells into prominent relief in contrast to their usual inconspicuous place in the tissue.

Three cases had some involvement of the spleen. In the central nervous system no changes were observed in the brain itself. In one case a good section was obtained of the choroid plexus. The ependymal cells showed vacuolization and slight hypertrophy as distinct from the normal regularity of the structure. These histologic changes were similar to

Fig. 2 Growth curves of rabbits on experimental diets.

those observed in chicks suffering from vitamin A deficiency (Phillips, '37). Myelin degeneration was found in the sciatic nerve of five animals and in the region of the brachial plexus in three. Slight alteration was noted in the optic nerve but its interpretation was doubtful. Three animals showed an alteration of the Purkinje cells of the cerebellum which was marked by atrophy, a homogenous cell change and areas of

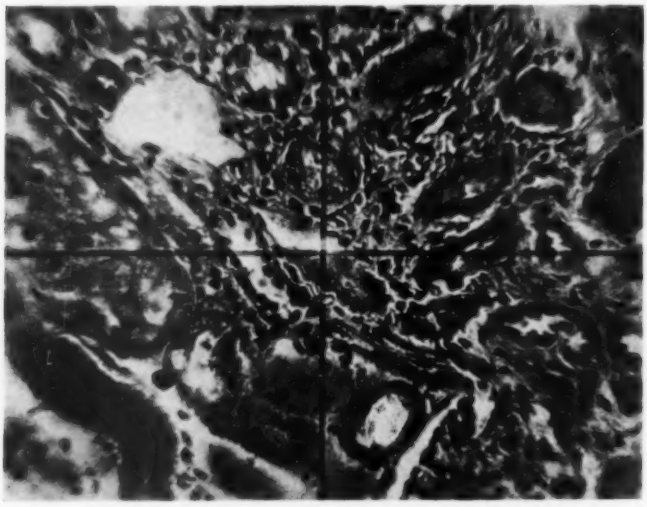

Fig. 3 Photomicrograph of an area of the kidney of a rabbit fed the basal ration only. Note the interstitial proliferation and parenchymatous degeneration. Magnification × 450.

cell disappearance. In the areas where the Purkinje cells had disappeared, Bergman cells were conspicuous.

Pregnancy and gestation were obtained in experiment no. 362 where carotene was added to the ration. No evidence of reproductive activity was observed on the basal ration alone or with additions of 2% Wesson oil, 2% aerated cod liver oil, or where the carotene was injected. Attempts to carry the females through a lactation period were not made.

Examination of the optic foramina showed little if any change remotely suggesting stenosis. Frequently the lacrimal glands were inflamed and swollen. At least one instance of secondary inflammation involving the maxillary bone was observed, and exostosis was found in the floor of the orbit which was suggestive of possible changes which might be anticipated in severe infections. In most cases the molar teeth of these rabbits lacking adequate amounts of vitamin A were unevenly worn.

DISCUSSION

It is apparent from these experiments that blindness from stenosis of the optic foramen cannot be attained in rabbits by the use of the Walker diet. In all other respects the syndrome developing in the rabbit from this ration low in vitamin A is analogous to that of calves. The symptomatology, behavior, growth curves, and ultimate course of the disease were alike. The ration was shown by Walker ('36) to contain from 9 to 10 mg. of carotene per 100 pounds. It seems that small quantities of vitamin A such as this ration contains permit physiological activity of low grade in the slow growing species. Thus variable manifestations may occur. It is possible that heavy sinus infection of the nasal passages in the case of the bovine might set up secondary inflammatory processes in the surrounding osseous tissue thus creating intra-osseous growth and pressure. This is not unlikely in the bovine where the sinusoidal spaces of the nasal passages very largely surround the eye. Further should this occur early in calfhood, permanent damage would result. It would be impossible to recover, even though vitamin A or carotene were given. Recovery from some of the symptoms was slow or nil in the cases where small remedial doses were tried. These experiments showed plainly that recovery from the developing syndrome was possible only where large overdosages of carotene or vitamin A were given.

The evidence obtained in these experiments indicates clearly that the primary deficiency of this ration is vitamin A in the case of the rabbit. If one or more deficiency factors are the causative agent or agents for the atypical blindness of cattle one must assume that the dietary requirements of the rabbit for the factor are either extremely low or unnecessary, or that the rabbit can synthesize it. Since excellent performance and even the added burden of pregnancy were obtained when ample carotene was administered it seems more logical to assume that the deficiency is an inadequate source of vitamin A. This is all the more likely since little is known concerning the various factors which condition the absorption and conversion of carotene into vitamin A and its utilization. Unpublished data of Phillips and Hart ('36) indicate that certain types of ration distinctly increase the vitamin A requirement of the chick when carotene is the sole source of the vitamin.

SUMMARY AND CONCLUSIONS

Experiments using a diet which caused optic foramen stenosis in calves failed to produce stenosis in the rabbit. The ration did produce a syndrome in the rabbit otherwise strikingly similar to that produced in calves. The effects upon growth, equilibrium, and the eye resembled the syndrome which produced stenosis of the optic foramen in calves. The development of this syndrome could be obviated by feeding adequate quantities of carotene or vitamin A. Fifty micrograms of carotene per kilogram of body weight afforded protection and the maintenance of health. Wesson oil, aerated cod liver oil, or a flavin concentrate were without the preventive or remedial effect. The remedial dose of carotene was distinctly above the preventive level of 50 micrograms per kilogram of body weight. Small doses, 30 to 70 micrograms per kilogram of body weight, were without remedial effect but if the dosage were increased from six to twenty times, recovery took place. One hundred per cent mortality was experienced unless a source of vitamin A was added.

These data indicate that an adequate source of vitamin A was the primary deficiency of this ration and that sub-minimal amounts of vitamin A may produce various physiological reactions which are the results of both primary and secondary tissue responses.

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THE MEASUREMENT OF THE EFFICIENCY OF DIETS. NEW APPARATUS AND PROCEDURES [1]

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FOUR FIGURES

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Within the memories of the middle-aged of today nutrition was understood to be a comparatively simple process, and nutritive values of foods were measured, with general confidence and satisfaction, in terms of calories, digestible nutrients, metabolizable energy or net energy.

During the current century, however, the science of nutrition has been, in effect, born anew—so great has been the advance in our knowledge; and, as each revolutionary discovery has followed the one before, thus contributing to our understanding of the subject as one of vast complexity, the idea of evaluating foods in terms of common measures has been pushed so far into the background as to appear to be ever less and less practicable and significant.

In reality, however, human society has not outgrown the need for measures of food value; we deal with foods in a quantitative way; foods are obtainable in only limited amounts; and nutritive values in relation to cost will remain always a consideration of first-class economic importance.

An especially baffling feature of this whole matter has been the growing appreciation, to which this laboratory has contributed a long series of studies, of the fact that foods cannot be scientifically evaluated, individually, in any common terms, since each essential nutrient, at least finally, if not immediately, affects the utilization of every other.

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